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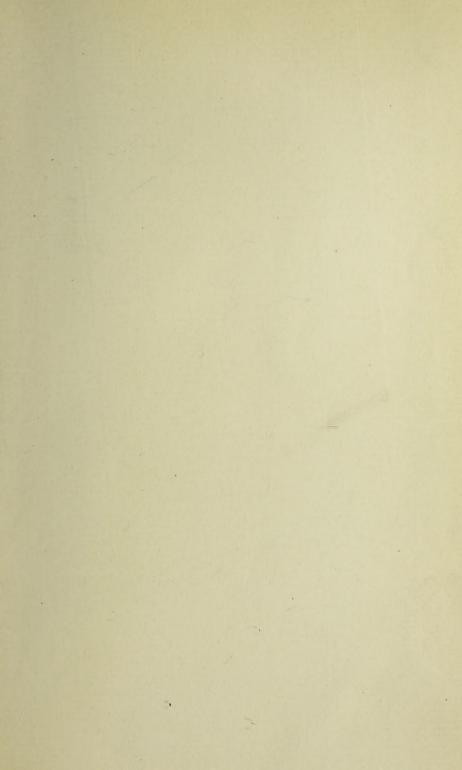
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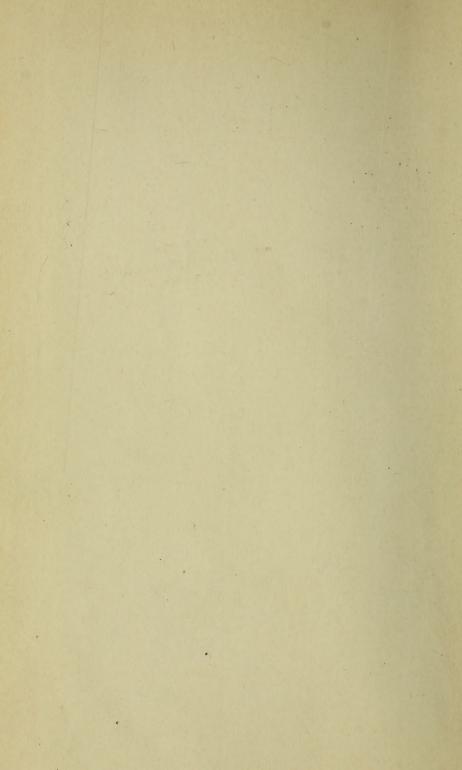
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DEPARTMENT OF THE INTERIOR BUREAU OF GOVERNMENT LABORATORIES BIOLOGICAL LABORATORY

Some Observations on the Biology of the Cholera Spirillum

BY

WM. B. WHERRY, M. D.

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LETTERS OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, September 20, 1904.

SIR: I have the honor to transmit herewith a paper by Dr. Wm. B. Wherry of the Biological Laboratory on "Some Observations on the Biology of the Cholera Spirillum."

I am, very respectfully,

Paul C. Freer,
Superintendent Government Laboratories.

Hon. Dean C. Worcester,

Secretary of the Interior, Manila, P. I.

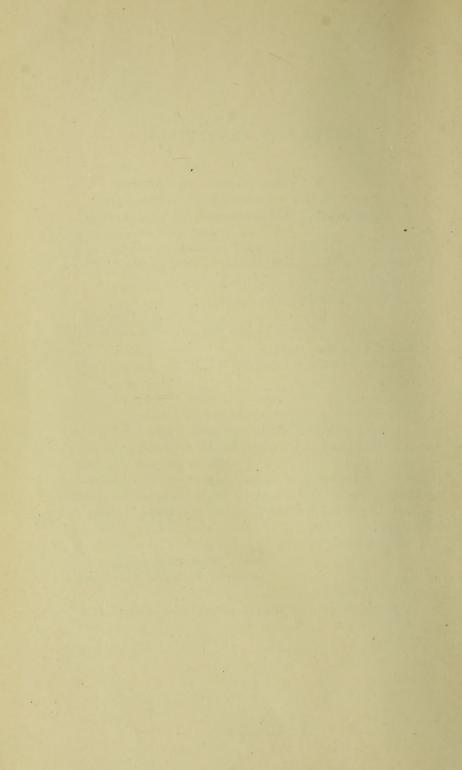
DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY, OFFICE OF THE DIRECTOR,
Manila, August 31, 1904.

SIR: I have the honor to transmit herewith and recommend for publication "Some Observations on the Biology of the Cholera Spirillum," by Wm. B. Wherry, M. D., Bacteriologist Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

Dr. Paul C. Freer,
Superintendent Government Laboratories, Manila, P. I.



SOME OBSERVATIONS ON THE BIOLOGY OF THE CHOLERA SPIRILLUM.

By WM. B. Wherry, M. D., Bacteriologist, Biological Laboratory.

INTRODUCTION.

The following observations were made during the past year while I was engaged in some studies preliminary to and in connection with the subject of toxin production.

Such marked variations in the morphology and biochemical characters of the cholera spirillum occurred during some earlier work that it was deemed advisable to adopt a modification of the methods of standardizing culture media, published as the "Procedures," etc., by the Committee of American Bacteriologists.¹

It is to be regretted, from a purely descriptive standpoint, that the organisms were not grown upon media prepared exactly according to the recommendations of the American Committee; but, since the main issue concerned the factors influencing toxin production, and since it was impossible to carry on two entirely separate sets of observations, a slight modification of these recommendations was employed for reasons which are given below.

Notwithstanding the use of these methods, a comparison of the biochemical peculiarities of the cultures chosen for this study reveals many points of difference between them—such as the production of a pellicle on bouillon by one, while another gives a diffuse cloudiness, the presence or absence of the cholera-red reaction, variations in the growth on potato, or in the type of the liquefaction of gelatin, etc. Many of these points of difference are

¹ The Reports and Papers of the American Public Health Association, 1898, XXIII, p. 60; or, for a brief summary of this report, vide L. Grimbert, on the Diagnosis of Bacteria by their Biochemical Functions, Arch. d. Parasitologie, 1903, VII, p. 304.

still emphasized in bacteriological literature, especially in the descriptions of single species. A careful preliminary study of one of these cholera cultures ("579") revealed such a wide variation in its morphology and in some of the details of its cultural characteristics, that I was forced to the conclusion that they could not be seriously considered in species description—since they are variations which will occur at intervals in the same culture.

It is hoped that this study, which was carried out under more uniform conditions than can be attained by older methods, will emphasize the variability of some bacteria and in a measure further the investigation of those factors entering into the production of such variations.

I have decided to present the subject-matter in the following order:

- A description of the method of preparing and neutralizing the media.
- II. The source, isolation, biochemical peculiarities, and varations of culture "579," with special reference to—
 - (a) The demonstration of the cholera-red reaction.
 - (b) The liquefaction of gelatin.
 - (c) The optimum reaction.
 - (d) The production of alkali.
- III. A description of the source and isolation of five other cholera cultures and of their resemblance to one another, and to culture "579."
- IV. Their growth in the presence of carbohydrates.
- V. Their relationship as shown by agglutinating and bactericidal sera.
- VI. Their pathogenicity.
- VII. Their morphology and pleomorphism.
- VIII. Summary and conclusions.

During this comparative study of a number of cultures from different sources, every precaution was taken to avoid contaminating one strain with portions from another culture, and the purity of each was controlled by frequent microscopical examinations and plating in gelatin or agar.

I. A DESCRIPTION OF THE METHOD OF PREPARING THE MEDIA.

One of the chief modifications of the methods recommended in the "Procedures" concerns the way in which the media was neutralized and the desired reaction obtained. The recommended method consists, briefly, in titrating a portion of the medium, as near the boiling point as possible, with phenolphthalein as an indicator; obtaining an accurate neutral point by the addition of normal sodium hydroxide to the bulk of the medium, and then adding sufficient normal hydrochloric acid to give the desired reaction. In this work the acidity or alkalinity to phenolphthalein was adjusted by the addition of normal sodium hydroxide alone, and unless otherwise stated the reaction refers to that established before sterilization. The term "final reaction" indicates the reaction of the medium after sterilization or just before its use. The sign (+) stands for acidity, while (—) indicates alkalinity. The figures are in per cent. Thus +1 means 1 per cent ¹ acid with phenolphthalein as an indicator (slightly alkaline to litmus).

This modification was adopted for the following reasons: Our préparateur made some bouillon in which the cholera spirillum would not grow at 35°-37°, whereas B. coli did so in it, luxuriantly, at that temperature. Upon investigating this phenomenon it was found that if, after neutralization with sodium hydroxide, the precipitated acid albumins were first filtered and then the hydrochloric added, the acid may exert a germicidal or inhibiting effect upon the cholera spirillum—the degree of inhibition apparently depending upon the thoroughness with which the acid albumins have been removed. Further, this inhibiting effect is more marked at body than at room temperature.

I have not been able to reproduce this phenomenon at every trial. Whether this is due to a variation in the amount of acid albumins actually removed on neutralization (it is well known that a portion of the precipitate formed by neutralization redissolves in an excess of alkali), or to some undetermined cause, the fact remains that such a fluid will sometimes completely inhibit the growth of the cholera spirillum, but not that of B. coli. It seems as if work with chemically pure solutions might furnish another biological proof of the existence of ionproteid compounds and also an instance of a specific (?) antitoxic action exerted by a proteid. This would not be the only example of such an antitoxic action, for, as shown by Kahlenberg and True, hydrogen and various metallic

¹One per cent acid or alkaline to phenolphthalein would, in chemical terms, represent 10 cubic centimeters of a normal acid or alkali to 1,000 cubic centimeters of the medium employed, or N/100.

² The Theory of Electrolytic Dissociation, H. C. Jones, 268-270. In my case, however, the action may possibly be due to free H ions from the hydrochloric acid, since it is a well-known fact that even small traces of acetic, hydrochloric, or citric acids completely destroy the growth of Spr. choleræ.

Kations exert a marked toxic action, at certain concentrations, on the germinating seedlings of *Lupinus albus*. The copper ion was found to be especially toxic and the seedlings barely lived in a solution containing a gram-molecular weight of copper ions in 51,200 liters of solution. But, when the copper ion was in combination with an organic complex, as in Fehling's solution, the roots would grow in a solution of this salt which contained a gram-atomic weight of copper in 400 liters.

The American committee does not recommend the removal of the acid albumins preliminary to the addition of the acid, but since Schultz-Schultzenstein ¹ has shown that in fluids containing albumin or peptone or both, 0.097—0.217 per cent of HCL will destroy the cholera spirillum in an hour; and since further, as demonstrated by Smith,² hydrochloric acid is destructive to diphtheria toxin; and again, since Ritchie ³ has pointed out a similar destructive action upon tetanus toxin, it was deemed advisable to leave the hydrogen ion out of the media.

The method of sterilization indicated in the "Procedures" was followed with the exception that bouillon and agar were sterilized in the autoclave at 120° for half an hour. This prolonged sterilization at a high temperature was found necessary and expedient on account of some very resistant spored organism encountered during the last hot season. It does not noticeably affect the nutritive qualities of agar or bouillon, although the initial acidity is raised about 0.5 per cent. When — 1 agar or bouillon is subjected to such an autoclaving a precipitate is usually thrown down. On filtration and further autoclaving the alkalinity is found to have been diminished by about 0.5 per cent.

This discrepancy, according to the "Procedures," "is perhaps due to side reactions which are not understood." The usual formation of a precipitate, which seems to vary in proportion to the amount of alkali added, with the simultaneous reduction in the alkalinity of the medium, seems to point to the hydrolysis and separation of insoluble compounds under the influence of the alkali.

¹ Schultz-Schultzenstein: Zur Kenntnis der Einwirkung des menschlichen Magensekrets auf Choleravibrionen. Cent. Bakt. 1st Abt., 1901, XXX, 785–790.

²Theobald Smith: The Relation of Dextrose to the Production of Toxine in Bouillon Cultures of the Diphtheria Bacillus. *Jour. of Exper. Med.*, 1899, IV, 383.

⁸J. Ritchie: Artificial Modifications of Toxins with Special Reference to Immunity. Journal of Hygiene, 1901, I, 130.

Contrary to a statement made in the footnote on page 72 of the "Procedures," I have several times noted the evolution of ammonia when bouillon is boiled after the addition of the fixed alkali. As might be expected, this is most marked in the case of fermented bouillon, as illustrated in the following instance:

Three thousand cubic centimeters of sugar-free bouillon had an initial reaction of +3.0; 2.5. per cent (75 cubic centimeters) normal sodium hydroxide was added and thoroughly mixed. This should have given a calculated reaction of +0.5. The solution, containing a dense flocculent precipitate, was boiled for two minutes. During ebullition an appreciable quantity of ammonia was given off which could be detected both by the odor and by the blueing of red litmus paper moistened with distilled water. The solution was filtered, brought up to 3,000 cubic centimeters by the addition of distilled water, and then showed a reaction of +1.0-i. e., the acidity had been raised 0.5 per cent over the calculated one.

A similar change occurs in unfermented meat extract, but here the escape of volatile alkali takes place more slowly.

II. THE SOURCE, ISOLATION, BIOCHEMICAL PECULIAR-ITIES, AND VARIATIONS OF CULTURE "579."

NECROPSY No. 579.

Emeterio Darita, age 28 years, male, Filipino, residence, Lorcha *Horatio* (boat on the Pasig River). Died April 17, 1903, after an illness of ten hours. Clinical diagnosis, cholera. Autopsy performed three and three-fourths hours after death.

It was learned that the man had numerous stools and considerable vomiting before death.

The body was that of a very muscular man. There was not much shrivelling of the skin. The face was in repose. The feet were in extreme flexion. The fingers were in forced flexion and could not be straightened. The skin of the palms, the soles of the feet, the fingers, and the toes was shrivelled, the tissue beneath it being dry and comparatively bloodless. On the soles of the feet were a number of blister-like elevations, varying from about 2 to 20 millimeters in diameter. These were found to be perfectly dry on section. The white of the eyes was icteric. The muscles were very firm. On section the body tissues appeared abnormally dry. The superficial glands were not enlarged.

The thoracic cavity contained no fluid. There were a few rather firm, fibrinous adhesions between the lungs and the anterior thoracic walls. Posteriorly, the lower part of both lungs was bound down

by rather firm, fibrinous adhesions. The thoracic organs were covered by a scanty adhesive secretion.

The *lungs* were somewhat emphysematous throughout. The *peri*cardial cavity contained a small amount of sticky secretion.

The heart was about normal in size and was in firm systole. It contained a considerable amount of thick, very dark, clotted blood. The blood which oozed from the larger arteries and coronary vessels was likewise of a very dark color and semicoagulated consistency. Otherwise the heart appeared normal. There was a persistent thymus gland about 5 by 15 millimeters in size.

The peritoneal cavity contained no fluid. The appendix was normal. The surface of the visceral and parietal peritoneum was covered with a scanty very adhesive secretion, which dried readily on exposure to the air. The parietal and visceral peritoneum throughout was of a rosy pink color.

The spleen was somewhat small in size and rather soft, and showed no particular changes on its surface or on section.

The gall bladder was filled with dark-green bile.

The *liver* was about normal size. The surface appeared somewhat cloudy, and on section the lobular markings were indistinct.

The *kidneys* were of about normal size; the capsules stripped readily, leaving a dark-red surface, on which the stellate veins stood out prominently. On section, the cortical and medullary markings were very indistinct.

The stomach contained a considerable amount of fluid substance, with many well-preserved rice granules. Its mucosa showed no particular change. The intestinal tract throughout contained a quantity of whitish, mucoid substance, which showed many white flocculi or rather flecks of a white albuminoid material. The secretion was very slippery while wet, but became very sticky on drying. The mucous surface of the duodenum and jejunum showed no change, but appeared rather whiter than normal. The mucosa of the upper two-thirds of the ileum showed no particular change, but a few of the solitary follicles were enlarged. In the lower third of the ileum the solitary follicles and Peyer's patches were quite generally enlarged, and some of the Peyer's patches appeared congested through the mucosa. The enlargement of the lymph follicles was most marked just above the ileo-cecal valve, where they were about 2 millimeters in diameter. In several places in the lower

portion of the ileum the mucosa was almost completely desquamated for a distance of several inches. The *pancreas* appeared rather pale on section.

The mesenteric glands were enlarged throughout to the size of almonds, and were pale on section (a common post-mortem finding here).

The *colon* and the upper portion of the *rectum* contained fluid contents similar to that described above. Here the mucous surface showed no particular changes, except that the solitary glands were enlarged—especially in its upper portion.

Anatomic Diagnosis.—Cholera; acute follicular and necrotic enteritis; follicular colitis; acute parenchymatous nephritis; acute parenchymatous hepatitis; adhesive pleuritis; emphysema of the lungs; persistent thymus.

Tissues from the organs hardened in Zenker's solution.

Smears.—Ileum (carbol fuchsin 1:10): Showed almost pure culture of slender rods, often curved, quite numerous; many degenerated, columnar epithelial cells. (See fig. 2.) Spleen (Gram and Safranin): No bacteria. Heart's blood (Gram and Safranin): No bacteria.

Method of isolation.—Cultures were made from the ileum according to the Schottelius enriching method. Peptone solutions (1 per cent Witte's peptone and 0.5 per cent NaCl in distilled water) with a reaction of +1, +0.5, neutral, and -1 were inoculated and kept at $35^{\circ}-37^{\circ}$.

In twenty hours the + 1 tube showed a dense layer of growth near the surface, with beginning pellicle formation. A hanging drop preparation from the surface showed short, actively motile curved rods—apparently in pure culture.

The + 0.5 and neutral tubes were well clouded, no pellicles.

The -1 tube was faintly clouded with a thin pellicle in the process of formation. It showed actively motile curved rods in the hanging drop.

The addition of ten drops of chemically pure sulphuric acid to each of the tubes gave a distinct indol reaction, which was most marked in the +1 tube.

Twenty per cent gelatin plates inoculated from the surface growth of the + 1 peptone tube were kept at 18°-28°. In twenty hours pinhead-sized areas of liquefaction were produced. These were cir-

cular, well defined, and contained motile masses of growth of a broken-up, refractile character.

A pure culture was obtained on +1 agar.

Biochemical peculiarities and variations.—On +1 agar a luxuriant dirty-white growth is seen in twenty-four hours at $35^{\circ}-37^{\circ}$. Later the edges become crenated—especially if the agar is somewhat dry. The condensation water is densely clouded. In old cultures spine-like processes may project from the edges of the growth, which is much more luxuriant on fresh, moist agar than on the same medium with a slightly dry surface. (For the optimum reaction see under "Liquefaction of gelatin.")

In + 1 bouillon the growth is somewhat variable. On one occasion the bouillon may be uniformly clouded; in the following, this is termed the "anaërobic type of growth." Again it may be clouded with a dense layer of growth near the surface, which soon forms a pellicle. Below this is termed the "aërobic type of growth." In a stained preparation from a forty-eight hour culture the organisms are thicker and more curved than from one in peptone solution grown under like conditions. The morphology varies with the reaction of the medium. The character of the growth is independent of the presence or absence of muscle sugar, and is apparently due to the predominance of the aërobic type of the organism on the one hand, or of the anaërobic type on the other. I came to term these aërobic and anaërobic types of growth as a matter of convenience, but a better theoretical explanation is furnished by assuming that the difference is due to a variation in the specific gravity of the bacterial cells. If a number of bouillon tubes be inoculated from an agar slant, and kept under like conditions, some will show the aërobic type while others will be uniformly clouded and may remain so or form a pellicle at a later date. The type of growth can be transmitted by further inoculations in bouillon, although, in the case of the anaërobic type, there is a tendency toward a cropping out of the aërobic one.

The production of a pellicle in fluid cultures.—As with the diphtheria bacillus, and other organisms, the habit of producing a pellicle in fluid cultures can be firmly established by transferring a portion thereof through a series of fluid cultures. "579 A" is one, which after being transplanted in this manner at intervals of three or four days for a couple of months, shows little or no ten-

dency to grow in the deeper parts of the bouillon, while a dense layer appears near the surface of the fluid and a pellicle is formed in much less time than when the training process was initiated, and the same result can be obtained much more rapidly by using the pellicle formed on a liquefied gelatin culture. The whole process is, in fact, one of artificial selection. Inoculations from such cultures are usually made from the upper layers of the fluid and hence a series of such inoculations yields an artificially selected race of organisms of low specific gravity.

So far as indol and alkali productions are concerned, there is no difference in the action of the aërobic and anaërobic type of organisms. (See alkali production.)

It is evident that the presence or absence of a pellicle in bouillon cultures is of little value in the differentiation of species.

Litmus milk is acidified and coagulated in forty-eight hours. (Control tubes remain sterile.) In about four days a firm clot is formed with separation of the whey and partial reduction of the litmus. The fermentation of lactose bouillon is evidence that this culture produces lactase.

+ 1 glucose bouillon is faintly clouded, but the growth occurs mostly at the bottom of the test tube as a stringy, viscous mass. There is no apparent increase after twenty-four hours. (See growth in the fermentation tube.)

On potato (unneutralized) the growth is variable, sometimes none appearing, or again a slight dirty yellowish one may be seen in three or four days. This variability is probably due to a difference in the acidity of the potatoes used.

Solidified ox serum is rapidly digested.

When grown anaërobically (pyrogallic acid method), in +1 glucose agar, growth appears along the line of inoculation, but the culture is no longer viable after the second anaërobic transplanting.

(a) ON THE DEMONSTRATION OF THE CHOLERA-RED REACTION.

Immediately after isolation from the body, this organism gave a pronounced cholera-red reaction upon the addition of ten drops of chemically-pure sulphuric acid to cultures grown in peptone solution during eighteen to twenty hours at 35°-37°. Since then, this reaction has only appeared at intervals—even in solutions

prepared from Witte's peptone, which had been set aside as "Proper for Indol."

All the cultures mentioned in this article have shown the same variation from time to time.

For some time peptone solutions of various reactions were used both in isolating the cholera spirillum at autopsy, from stools, water, etc., and in testing for cholera red, but without any constant results which might determine whether any one reaction favors surface growth or the demonstration of the reaction. Dunham's peptone solution containing 1 per cent Witte's peptone and 0.5 per cent sodium chloride in distilled water has a final reaction of +0.5, and has given the best results on the whole. This solution has such poor nutritive qualities for many species of intestinal bacteria that it is especially suitable for isolation by the Schottelius enriching method.

Upon investigating this uncertainty of the cholera-red reaction, I determined to try sugar-free bouillon, which has been shown by Smith ² to be such an excellent culture fluid for the production of indol by bacteria. In the first batch of this medium, these cultures gave excellent cholera-red reactions, but in two subsequent ones the reaction failed to appear. These three media were shown to be free from nitrites and fermentable sugars, by testing with *B. coli*, as recommended by Smith. In addition to sugar-free bouillon, four different peptone solutions were tested, namely: *Peptone Sicca cum Sale*, R. Nishiyama, Osaka, Japan; *Peptone Carne*, E. Merk, Darmstadt; and two samples of *Peptonum Siccum*, Friedr. Witte, Rostock—one of which had been marked "Proper for Indol." These too were found to be free from fermentable sugars and nitrites, but all failed to yield cholera red. However, they gave the indol reaction upon the addition of a trace of sodium nitrite.

As is well known, the demonstration of the cholera-red reaction depends upon the fact that an organism not only forms indol, but

¹ It may be noted that this so-called "peptone" consists of a mixture of albumoses and contains only a minimal quantity of true peptone—Torald Sollmann, Witte's Peptone: Its Dissociation, and its Combination with Acid and Alkali. Amer. Jour. of Physiol., 1902, VII, 203; on the other hand, there are "peptones" on the market, such as that manufactured by the firm of Chapoteau, which contain as much as 50 per cent of pure peptone (J. P. Pawlow: The Work of the Digestive Glands, 1902, 96).

² Theobald Smith: A Modification of the Method for Determining the Production of Indol by Bacteria. *Jour. of Exper. Med.*, 1897, II, 543-547.

also either produces nitrites or reduces nitrates to nitrites. Having a premonition that the inconsistancy of the reaction might depend upon a variation in the amount of nitrates present in different lots of peptone or meat extracts, or upon their accidental introduction on one occasion and not on another (when Cross and Blackwell's table salt is used, much more constant results are obtained, than when C. P. sodium chloride is employed), I prepared peptone solutions in the manner above indicated, but in addition to the C. P. sodium chloride, I introduced 0.01 per cent C. P. sodium nitrate (1 cubic centimeter of a 10 per cent solution per liter). In such a solution the cholera-red reaction is not only constant, but it appears more promptly and is more intense than usual. Control peptone solutions, not containing sodium nitrate, failed to give the reaction. (All the cholera cultures mentioned in this article give the reaction constantly and promptly in this medium.)

Since completing this work, I have found that Max Bleisch, as long ago as 1893, emphasized the necessity of introducing nitrates into the peptone solution.

There seems to be some evidence that the nitrate content of meat extract or "peptone" may vary; and this may account for some of the discrepancies in species description, for an organism attributed with nitrifying powers may only possess the ability to reduce nitrates to nitrites—a property common to many species of bacteria.

Thus, to cite an instance: Last year Woolley and Jobling, working in this laboratory, described cultures of *B. bovisepticus* which gave a distinct cholera-red reaction upon the addition of chemically-pure sulphuric acid to cultures grown for from twenty-four to thirty-six hours in Dunham's peptone solution. At that time the cholera cultures here mentioned also gave the same reaction. I have recently tested two of these cultures of *B. bovisepticus*, and find that they fail to give the cholera-red reaction in the peptone solution in which the cholera cultures fail to do so, but yield it promptly when grown in the peptone solution containing 0.01 per cent sodium nitrate.

As shown by Kastle and Elvone,3 hyponitrous acid, nitrous acid, nitrites,

¹ Max Bleisch: Ueber einige Fehlerquellen bei Anstellung der Cholerarothreaktion und ihre Vermeidung, Zeit. für Hyg. und Infekt., 1893, XIV, 103-115.

² P. G. Woolley and J. W. Jobling: A report on Hemorrhagic Septicemia in Animals in the Philippine Islands, *Bull. No. 9*, *Biological Laboratory*, *Bureau of Government Laboratories*, p. 8.

³ Kastle and Elvove: Oxidation and Reduction in the Animal Organism and the Toxic Action of Powerful Oxidizing and Reducing Substances. *Amer. Chem. Jour.*, 1904, 31, 195–207.

etc., are, in part, converted into nitrates in the animal body. And as has been demonstrated by Mayo,¹ when potassium nitrate is fed to cattle, a chemical test for nitrates can sometimes be obtained after their death, although, as a rule, nitrates are rapidly reduced to nitrites in the tissue fluids.

It seems probable that the use of Smith's sugar-free bouillon, containing 0.01 per cent sodium nitrate, would furnish a means of testing the production of indol and the simultaneous reduction of nitrates by many bacteria.

(b) ON THE LIQUEFACTION OF GELATIN.

At the time of isolation, this organism showed active proteolytic properties—liquefaction of 20 per cent gelatin appearing within twenty-four hours, at 18° to 28°, and rapidly spreading to the sides of the test tube as a shallow, circular, pan-shaped area. Then the liquefaction descended progressively from above downwards, involving the whole width of the tube, with a slight funned-shaped depression in the center along the needle puncture. Careful data concerning the reaction and dryness of the gelatin were not kept at the time, but some variations in the rate and character of liquefaction were noticed. Further work at the time being impossible, the original agar culture was kept in the ice chest (transplants on +1agar being made at intervals of every two months) for eight months. The organism still showed the above type of liquefaction (which is often described as being characteristic of Spirillum Finkler Prior) at $18^{\circ}-28^{\circ}$ in 20 per cent gelatin, which had a final reaction of +1.2. At the same temperature, in fresh 20-per cent gelatin, which had a final reaction of +2, the organism slowly produced, in the course of three days, a small turnip-shaped area of liquefaction which, drying at the surface, left a small bubble-like depression—that is, it produced the type of liquefaction which was described by Koch as being characteristic of the cholera spirillum.

In two separate trials with the same gelatin (+2) at 35°-37°, the inoculated material precipitated, and no growth or liquefaction occurred.

In +1.5 gelatin at $10^{\circ}-15^{\circ}$ no growth occurred in ten days, but rapid liquefaction took place on change to $18^{\circ}-25^{\circ}$.

When grown anaërobically (pyrogallic acid method) at 18°-28°

¹N. S. Mayo: Cattle Poisoning by Nitrate of Potash, *Bull. No. 49* (1895), Kansas State Agricultural College.

in +1 gelatin containing muscle sugar, growth appears along the stab but no liquefaction takes place in three days.

Before detailing some experiments performed to determine the factors influencing variation in the type of liquefaction, it may be well to note some of the points brought out in the literature on this subject.

The proteolytic ferments of bacteria are only active in a medium alkaline to litmus, and it takes but a small amount of acid to hinder their action. This is in accord with the behavior of trypsin. When carbohydrates which can be so fermented as to form acids are present in gelatin, its liquefaction is inhibited. In 1898 Auerbach, working with a number of liquefying bacteria, showed that the inhibiting power of glucose exceeded that of lactose, and that in the case of *B. vulgare* the acid products of fermentation inhibited the formation of the ferment itself. It seems that for the production of the ferment a medium containing albumin and the access of free oxygen is necessary. According to Liborius² liquefaction of gelatin takes place very slowly in the absence of oxygen—with the exception of the case of some anaërobes.

According to T. Sollman (loc. cit, p. 211), "Kühne investigated the action of B. subtilis and B. prodigiosus on solutions of protalbumose from the chemical standpoint, and found that the phenomena resemble closely those of tryptic digestion. The conversion to tyrosin, leucin, and tryptophan was often almost complete." Again, according to Gotschlich, "Kalischer in experiments to determine how much of the casein splitting was due to the ferment and how much to the living cells, found that the ferment was able to produce peptone, leucin, tyrosin, as well as ammonia and aromatic oxyacids—in which its action also is in harmony with that of trypsin."

The melting point of gelatin undoubtedly plays a part in influencing the type of liquefaction which will occur at any given temperature. In my own experience the addition of alkali lowers the melting point. Thus, neutral gelatin which will not congeal at $18^{\circ}-28^{\circ}$ will do so in the ice chest, and +1 gelatin is not as solid as +1.5 gelatin at the same temperature. An interesting communication by Paul von Schroeder throws some light on this subject. "When a gelatin solution is heated at 100° , and samples are taken out at intervals and placed in a thermostat at 25° ,

¹Auerbach: Ueber die Ursache der Hammung der Gelatinverflüssigung durch Bakterien durch Zuckerzusatz. Ref. C. B., II Abt. 1898, IV, 492–494.

²Liborius: Beitrage Zur Kenntniss des Sauerstoffbedürfnisses der Bacterien, Ztschr. f. Hyg., 1886, I, 115–176.

³E. Gotschlich: Handbuch der Pathogenen Mikroorganismen, Kolle u. Wassermann, 1903, I, 107.

⁴Paul von Schroeder: Phenomena of the Setting and Swelling of Gelatin, Review: *Jour. of the Chem. Soc'y*, 1903, vol. 84, ii, 721.

their viscosity being determined five minutes later, it is found that the values of the viscosity diminish, as the duration of the heating at 100° increases, ultimately becoming constant.

"This change is attributed to a process of hydrolysis * * *.

"Certain salts increase the viscosity, magnesium salts exerting the greatest influence * * *.

"The effect of hydrochloric acid and sodium hydroxide on the behavior of gelatin solutions was similarly studied. The process of hydrolysis is accelerated by both hydrogen and hydroxyl ions, and the final value of the viscosity thus attained after hydrolysis is lower than that reached in pure or salt containing gelatin solutions."

Again, according to Rousseau, if gelatin be dialized, so as to remove the calcium salts contained therein, one obtains a solution which, sterilized in an autoclave at 120° for twenty to thirty minutes, solidifies upon cooling.

In order to determine what influence the reaction and dryness of the gelatin exert upon the type of liquefaction produced by a given cholera culture, the following experiment was performed: Nutrient gelatin was prepared containing 20 per cent gold label gelatin, 1 per cent Witte's peptone and 0.3 per cent Liebig's beef extract. It was divided into halves and to each portion normal NaOH was added, one-half receiving more than the other. After sterilization one portion showed a final reaction of +0.8, while that of the other was +1.0. In addition to this, another sample of gelatin, slightly darker in color but prepared in the same way, which had been kept on ice for three weeks and showed some evaporation and a final reaction of +1.5, was used. This one was melted and resolidified before inoculation. Each sample contained a small amount of muscle sugar as shown by subsequent fermentation with $B.\ coli$.

Four tubes from each of these samples were then inoculated from a twenty-four-hour culture of "579" on +1 agar, which had been kept on agar transplants at 35°-37° for two and a half months. In forty-eight hours at 18°-28° there was quite a noticeable variation in the amount of liquefaction produced in the different sets of tubes. The amount of this, in the four tubes of any one of the three sets, was not exactly uniform, probably on account of a variation in the number of bacteria introduced at the time of inoculation,

¹Em. Rousseau: Influence of the Salts of Calcium upon the Solidification of Gelatin Sterilized at 120°, Bull. Inst. Pasteur, 1903, I, 719.

but the difference between the three sets was very noticeable. Any one of the four +0.8 tubes showed more advanced liquefaction than any of the +1 tubes, and the difference between the +1 and +1.5 tubes was still more marked, as shown in fig. 1.

It is often stated that "bacterial proteolytic enzymes, like trypsin, show increased activity in the presence of certain chemicals, such as sodium carbonate and salicylate."

So far as the action of certain ions upon the tryptic digestion of fibrin is concerned, A. Kanitz, in reviewing the work of Dietz and confirming the quantitative experiments of Shields, has shown that the optimum concentration of the hydroxyls from barium, strontium, and calcium hydroxides varies between $\frac{1}{70}$ and $\frac{1}{150}$ of the gram-molecule per liter. Determination of the electric conductivity and other physical constants shows that these alkaline earths are strongly and almost equally dissociated at these dilutions, and since the three hydroxides work at the same concentration he concludes that the kation is without influence and that the anion is alone active. He then calculated, from the per cent of hydrolysis of potassium carbonate in given dilutions, the concentration at which the carbonate of potassium exerted the most active influence on tryptic digestion and found this to be about $\frac{1}{200}$ of the grammolecule per liter. He was unable to say that there was any difference in the mode of action of carbonate of potassium and the hydroxides of the alkaline earths. Kanitz concludes that the optimum for tryptic digestion is a liquid containing $\frac{1}{70}$ to $\frac{1}{200}$ of the hydroxyl ion (OH=17 gms.) per liter.

In order to test the above statement from a bacteriological standpoint, an experiment was performed as follows: I prepared one liter of nutrient gelatin by adding 20 per cent Gold Label gelatin, 1 per cent Witte's peptone, and 0.5 per cent sodium chloride to 1,000 cubic centimeters of distilled water; the ingredients were then dissolved by boiling; distilled water added to 1,000 cubic centimeters; the mixture then was divided into two parts of exactly 500 cubic centimeters each; each half titrated to phenolphthalein, and sufficient normal NaOH added to one-half to give a reaction of +1,

¹A. Kanitz: Ueber den Einfluss der Hydroxylionen auf die tryptische Verdauung. Zeit. für physiol. Chemie., 1902, 37, 75-80.

² John Shields: Ueber Hydrolyse in wässerigen Salzlösungen. Zeit. f. physikalische Chemie., 1893, 12, 167.

and an equal volume of normal Na₂Co₃ was then added to the other half; after cooling to 40° the whites of three eggs were added to each half; each portion was then boiled for three minutes, filtered through cotton, distributed and sterilized in the Arnold for twenty minutes on each of three successive days. The final reaction of the NaOH gelatin was +1.7, while that of the Na₂Co₃ gelatin was +1.8.

Six tubes of each were then inoculated from the same place on the edge of the growth of a twenty-four-hour agar culture of "579," and kept at 18°-28°. Liquefaction commenced and progressed slowly but equally during several days' observation.

The results of Kanitz would not appear to give conclusive proof of his view that the Kation exerts no influence on the optimum reaction, as a glance at the table given in his paper will show, the variations between the three alkaline earths being quite marked at different temperatures. In Dr. Wherry's work he compared equivalent amounts of soldium hydroxide and sodium carbonate, and thus, while he had the same concentration of hydroxyl ions, he had, in the case of the latter reagent, twenty-five times the number of sodions present in unit volume. This latter fact would tend to show that the kation is without marked influence; at least in the case of sodions. However, these results show that the question is one which is barely touched and is well worthy of complete investigation by a use of the methods of physical chemistry in biology.—FREER.

An attempt was made to increase the proteolytic activity of this culture by transferring it at intervals of every few days from one gelatin tube to another. At the end of four months this culture showed no greater activity than another transplant of the same culture which had been kept on agar slants for the same length of time.

What has already been said concerning the influence of the reaction of the medium upon the rapidity with which the cholera spirillum is able to liquefy gelatin has a direct bearing upon the type of liquified areas it will produce in or upon gelatin plates. Further, when, as has been noted by many observers, the same culture gives rise to two distinct types of liquified areas, the difference may be explained by the relation of the plated organisms to their oxygen supply. Thus, an organism situated at the surface on account of its greater supply of free oxygen might be expected to produce a more rapidly spreading area of liquefaction than one more deeply situated, where the supply is relatively less. Moreover,

the colony at the surface would be of the shallow, turbid type with a greater area than that of the deeper colony where the organisms encounter a greater resistance of the surrounding gelatin, and in consequence of which they would be massed together—producing the refractile "ground-glass" type of colony. It has been noted that such "ground-glass" colonies, upon further growth, invariably break up into liquid areas with turbid contents and such breaking up occurs pari passu with a lessening of the surrounding resistance and an increase in the supply of free oxygen. That such a supply of free oxygen does exist in a thin layer of gelatin can be proven by covering the opening of a gelatin stab culture with a few drops of liquid gelatin. Here no liquefaction occurs until the organisms have spread nearly to the surface.

(c) ON THE OPTIMUM REACTION.

All the cultures mentioned in this article show much more luxuriant growth in eighteen to twenty hours, at $35^{\circ}-37^{\circ}$, on fresh -0.5 than on fresh +0.5 agar. Furthermore, the maximum amount of growth is obtained on -0.5 agar in eighteen to twenty hours, while that on +0.5 agar does not equal it in thirty-six to forty-eight hours at the same temperature. The -0.5 agar was prepared from Liebig's beef extract and had an initial acidity of 1 per cent acid to phenolphthalein. It was neutralized and brought to a reaction of -1. After sterilization, the reaction was reduced to -0.5. Since 20 cubic centimeters of normal NaOH were added in the first place, and part of this was lost in the precipitate thrown down by autoclaving, it contained between 1/50 and 1/100 of a gram-molecule per liter. This would seem to support the idea that the optimum conditions for the growth and multiplication of the cholera spirillum are such as will favor its proteolytic activity.

(d) ON THE PRODUCTION OF ALKALI.

Fifty cubic centimeters of Smith's sugar-free bouillon was placed in each of five 600-cubic centimeter Ehrlenmeyer flasks and autoclaved at 120°. Final reaction, +1.5.

Each was then inoculated with one loop from an eighteen-hour

¹For the method of preparation see Jour. of Exper. Med., 1899, IV, 375.

evenly clouded sugar-free bouillon culture of "579," and the following table illustrates the rate of alkali production:

No. of flask.	Tem- pera- ture.	Age of culture.	Reaction to phenolph-thalein.	Remarks.
	0	Hours.		
1	30-35	24	+0.8	Dense cloudiness; no pel- licle.
2	30-35	48	+1.0	Dense cloudiness; no pellicle; actively motile curved rods.
3	30-35	72	+0.2	Do.
4 5	30-35 30-35	96 120	Neutral. -0.8	Do. Dense cloudiness; no pel-
	00-30	120	-0.0	licle; rods not so actively motile; few curved filaments.

Alkali production progresses equally as well when a pellicle is formed. It also occurred in unneutralized sugar-free bouillon with an initial acidity of +2.3. If the sodium chloride usually added to sugar-free bouillon be left out, no formation of alkali can be detected by titration with phenolphthalein—at least during five days. In control flasks of the same bouillon, plus sodium chloride, alkali production occurred about as rapidly as shown in the above table. This would seem to indicate that the alkali is produced by a conversion of NaCl into NaOH or Na₂Co₃, and that the greater part of the alkalinity is owing to the formation of such substances rather than to ammonia, amine, and ammonium bases, to which it is usually attributed. However, it is also possible that sodium chloride exerts a catalytic (accelerating) action on the formation of ammonia.

III. A DESCRIPTION OF THE SOURCE AND ISOLATION OF FIVE OTHER CHOLERA CULTURES, AND OF THEIR RESEMBLANCE TO ONE ANOTHER AND TO CULTURE "579.

Cholera "Scout" is a culture obtained by the Schottelius enriching method from a stool sent to the laboratory from Caloocan on April 16, 1903. The patient was a native scout who died on the next day with typical symptons of Asiatic cholera, which was epidemic in Caloocan and the surrounding country at the time. In its cultural characteristics it is indistinguishable from "579," excepting that litmus milk is acidified in twenty-four hours at 37°; but no coagulation occurs in five days. Hence, like "579," it

produces lactase, and differs from it in the absence of the production of rennin.

Cholera "561" is a culture obtained by the Schottelius enriching method from Eugenia Holandes, a Filipina, 33 years old, who died on March 26, 1903, after an illness of three days. Autopsy, seven hours after death. To briefly summarize the post-mortem findings: The skin of the hands and feet is shrivelled; the feet are in extreme flexion with the toes in extension; the pleural and peritoneal membranes covered with a sticky secretion. Cloudy swelling of the solid organs. Ileum congested, especially in its lower half, the mucosa showing some patches of epithelial desquamation. Contents of ileum greenish black and containing much mucus. Old and advanced amebic colitis. Culturally it is indistinguishable from Cholera "Scout."

Cholera "A" is a culture obtained at autopsy by Dr. R. P. Strong some time in the fall of 1903, cholera being endemic in Manila at the time. Culturally it is indistinguishable from cholera "Scout."

Cholera "City Moat" is a culture obtained by Mr. Lindquist, of the First Reserve Hospital laboratory, from the city moat near the hospital, about July, 1903. Cholera was endemic in Manila at the time. Culturally it is indistinguishable from cholera "Scout."

Cholera "Pfeiffer" is a culture of that name brought by Dr. R. P. Strong from Germany. It has been grown on artificial media for a period of nine years, and during the past year has not been passed through animals. Culturally it is indistinguishable from cholera "Scout," but it is very much more sensitive to the action of agglutinating sera.

"554-B" is a culture obtained on March 20, 1903, from a cholera autopsy. Morphologically it appears as short, curved, actively motile rods. It closely resembles the above cultures but does not agglutinate with the serum of a rabbit immunized against "579" nor with the serum of a cholera convalescent.

IV. THEIR GROWTH IN THE FERMENTATION TUBE IN THE PRESENCE OF CARBOHYDRATES.

Medium: Smith's sugar-free bouillon, which had a final reaction of +1.5 containing 1 per cent of glucose, maltose, saccharose, and lactose. One per cent of starch was added to some of the same bouillon and autoclaved after distribution; the initial reaction was

not changed. When inoculated with "579" and kept at $35^{\circ}-37^{\circ}$ the following results were noted:

Bouillon.	Gas.	Reaction of contents of bulb and neck on fourth day.	Gas in control tubes inoculated with B. coli (fourth day).	Remarks.
Glucose	0	+3.3	30 per cent; $\frac{\mathbf{H}}{\text{CO}_2} = \frac{2.5}{1}$	Maximum growth attained intwenty-four hours. Bulb and closed branch turbid; no pellicle. Agar slants inceulated after twenty-four hours from the bulb or neck re-
Maltose	0	+3.2	45 per cent; $\frac{H}{GO_2} = \frac{3}{1}$	main sterile. Maximum growth attained in twenty- four hours. Bulb and closed branch turbid; no pellicle. Agar slants inocu- lated on the fourth day from the bulb or neck remain sterile.
Saccharose	0	+3.5	No gas; closed arm cloudy.	Do.
Lactose	0	+4.0	40 per cent; $\frac{H}{CO_2} = \frac{2}{1}$	Maximum growth attained in twenty-four hours. Bulb and neck turbid, ciosed branch clear; no pellicle. Growth more dense than in other sugars. Agar slants inoculated on the fourth day from bulb or neck show a luxuriant growth in twenty-four hours at 37°; activals.
Starch ¹	0	+4.0	No gas; closed arm cloudy.	actively motile curved rods in the hanging drop. Maximum growth attained after twenty-four hours. Culture viable on fourth day as per lactose tube.

 $^{^{1}\}mathrm{A}$ test tube containing the same starch solution became densely turbid and a well-marked pellicle was formed. On the fourth day the acidity had reached 3 per cent of a fermentation tube was filled with this culture and -1 bouillon added. When inoculated with B. coli 30 per cent of gas was formed in forty-eight hours $\frac{H}{\mathrm{CO}_2} = \frac{3}{1}$.

It will be noticed that in glucose, maltose, and saccharose bouillon there was growth in the closed arm as well as in the bulb, and that the acids produced were of such a character as to destroy the vitality of the organism. On the other hand, in the case of the lactose and starch bouillon, no growth occurred in the closed arm, and, although a greater quantity of acid was produced, the organism was still viable on the fourth day.

In another series of experiments, in which 0.5–1 per cent glucose bouillon (final reaction = +1.5) was distributed in small flasks and inoculated from the same culture and kept at $35^{\circ}-37^{\circ}$, the maximum amount of acid (3–3.5 per cent) was produced in twenty-four hours and transplants made at that time remained sterile.¹

Again, enough normal NaOH was added to sugar-free bouillon to give a calculated neutral reaction. The final reaction after autoclaving was +0.7. A sterile solution of glucose, amounting to $\frac{1}{40}$ per cent, was then added and the flask inoculated and kept at 28° . In four days the acidity had been raised 0.5 per cent and the culture was still viable. The experiment was not carried on for a sufficient length of time to note whether the acid produced would be finally neutralized by such alkali production as normally takes place in sugar-free bouillon, but this is hardly probable as the growth, in the presence of even such a small per cent of glucose, is rapidly precipitated and forms a very viscous sediment.

The other cultures grown in solutions of these carbohydrates (reaction +1-+1.5) yielded similar results as shown in the following table:

Bouillon.	"Scout."	''561.''	"A."	"City moat."	"Pfeif- fer."	Remarks.
Glucose	+3.8	+3.6	+3.8	+3.5	+3.5	Titration on fourth day. Character of growth and fate of culture as per culture "579."
Maltose Saccharose Lactose Starch	$\begin{array}{r} +4.0 \\ +3.0 \\ +3.8 \\ +2.8 \end{array}$	$ \begin{array}{r} +4.0 \\ +3.0 \\ +4.0 \\ +3.0 \end{array} $	+3.0 +3.0 +4.5 +3.0	$ \begin{array}{r} +4.0 \\ +3.0 \\ +4.3 \\ +2.3 \end{array} $	$ \begin{array}{r} +4.4 \\ +3.0 \\ +4.0 \\ +2.8 \end{array} $	Do. Do. Do. Do.

Buxton,² in an excellent discussion on bacterial enzymes states that "cholera then produces amylase, maltase, but no invertase,

¹ See analogy in the case of the diphtheria bacillus (Th. Smith, loc. cit., p. 382). It is extremely probable that any toxin formed by the cholera spirillum would be destroyed in a manner similar to that which takes place in diphtheria cultures.

² Buxton: Mycotic Enzymes. Am. Med., July 25, 1903, 138.

lactase, nor inulase." These cultures seem to produce both lactase and invertase. The sugars used were prepared by Merk.

E. Gotschlich (loc. cit., p. 106) states that Fermi and Montesano found that invertin occurred inconstantly in the cholera spirillum and spirillum of Metchnikoff.

V. THEIR RELATIONSHIP, AS SHOWN BY AGGLUTI-NATING AND BACTERICIDAL SERA.

In applying the Gruber-Durham test to the study of the identity or relationship of the following cultures, a number of facts observed by others influenced both the choice of the method employed and the interpretation of the results.

In making a quantitative determination of the power of a given serum to produce a complete agglutinate when tested on a series of cultures, probably no one factor will influence the production of discordant results so much as quantitative variations between the agglutinin and the agglutinable substance. Thus, to cite an instance, an emulsion of culture "579" in 0.8 per cent sodium chloride solution was tested against the serum of a cholera victim diluted 1:100; agglutination was partial in thirty minutes and not complete until sixty minutes at 28°. The same emulsion was diluted with an equal quantity of the salt solution and then at 1:100 gave a complete reaction in thirty minutes at the same temperature.

A dense suspension of a culture when mixed with a powerful serum at a low dilution may give a prompt but only a partial reaction—numerous bacilli remaining unaffected in the serum which is now freed from agglutinins by the precipitated bacteria. On the other hand, as the dilution of the serum is increased, a similar disproportion is produced with the same result.

On account of variations in the density of the growth in bouillon, which the cultures studied at times show, emulsions of the bacilli in 0.8 per cent sodium chloride solution were exclusively employed. The cultures were grown on +1 agar for eighteen to twenty hours at 35°-37°, and the emulsions made to correspond as nearly as possible with the density of a twenty-four-hour typhoid culture according to the method employed by Smith ¹ in the comparative

¹Theobald Smith: Jour. of Exper. Med., 1898, III, p. 465.

study of tubercle bacilli. They were allowed to stand for ten minutes in order to give time for the coarser particles to settle. Such an emulsion is microscopically free from clumps, and the rods retain their active motility in the control drops for an hour or more.

The serum was diluted with 0.8 per cent sodium chloride solution in Thoma-Zeiss blood pipettes, and a loopful of this serum was then mixed with an equal quantity of the emulsion and examined from time to time with the 1/6 objective. It will be noted that the dilution of the serum in the drop was always twice that in the diluting pipette. Control hanging drops of the emulsion were always made and examined before and at the close of each experiment. The microscopic method was employed because it was believed that the end of the reaction can be more accurately determined and any differences in the character of the clumps noted.

It is a well-known fact that organisms, which have been grown for a long time upon artificial media, are more sensitive to the action of homologous sera than they are when their pathogenicity has been raised. Typhoid cultures recently isolated from the body sometimes show a marked resistance to agglutination with the patient's serum as compared with old laboratory cultures. As shown by F. Hamburger ¹ the agglutinability of cholera cultures diminishes with an increase in virulence.

AGGLUTINATION WITH THE SERUM OF A CHOLERA VICTIM.

The history of the serum is briefly as follows: Candido Nugin, a Filipino, 19 years old, died at the San Lazaro Cholera Hospital on January 8, 1904. He was ill for thirteen days; had rice-water stools during the acute stage of the disease, passed into the typhoid stage, and died on the thirteenth day with symptoms of acute nephritis. At the autopsy six hours after death, the kidneys showed acute parenchymatous nephritis; there was cloudy swelling of the liver and heart muscle. The ileum was still in a congested state, but its mucosa was in fairly good condition. Smears from the ileum showed a number of thin curved rods, mixed with many other organisms. No cultures were made.

The following table shows the agglutinating action of the serum from the hearts blood of this patient in such dilutions as were tested.

¹F. Hamburger: Wien. Klin. Woch., 1903, XVI, 97-98.

An accident to the serum prevented the determination of its agglutinating limit:

Culture.	Temper- ature.	Dilution of serum.	Result.
	0		
"579"	28	100	Small motile clumps in seventeen minutes; complete in thirty minutes.
"579"	28	200	Small motile clumps in ten minutes; complete in forty minutes.
"Seout"	28	100	Almost complete in thirty minutes; complete in sixty minutes.
"561"	28	100	Do.
"A"		- 100	Almost complete in thirty minutes; not complete in sixty minutes.
"City moat"	28	100	Complete in thirty min-
"Pfeiffer"	28	100	Complete in twenty min-
"554b"	28	1 4 0	Negative during an hour's observation.

My own serum diluted 1:20 produced no agglutination during forty-five minutes' observation.

In this experiment no attempt was made to use salt-solution suspensions of equal density and the variation in the time when complete agglutination occurred is noticeable.

AGGLUTINATION WITH IMMUNE RABBIT SERUM.

A rabbit was injected with 0.8 per cent sodium chloride suspensions of culture "579" grown on +1 agar for twenty-four hours at 35°-37°. It received the contents of about six agar slants subcutaneously and intraperitoneally during two months. In this twenty-four-hour-old serum the agglutinating limit is not great, but is considered sufficient for the following comparative and quantitative estimations:

Culture.	Temper- ature.	Dilution of serum.	Result.	Remarks.
"579"	25	800	+	Complete in thirty-five minutes; small, com-
"579"	25	2000	-	pact clumps. ¹ Partial in twenty-five minutes; not complete in
"Scout"	28	800	+	sixty minutes.¹ Complete in thirty-five minutes; small, loose
"561"	28	800	+	clumps. ¹ Do. ¹

¹The hanging drop was not examined during the five minutes previous to the given time, hence it is probable that the table indicates a greater uniformity in this respect than occurred in reality. (See footnote under "Morphology and pleomorphism.")

Culture.	Temper- ature.	Dilution of serum.	Result.	Remarks.
"A"	28	\$ 100	+	Complete in thirty-five minutes; small, compact clumps. ¹
"City moat"	28	800	+	Complete in thirty min-
"Pfeiffer"	28	800	+	utes.¹ Complete in thirty-five minutes; small, loose clumps.¹
"554b"	28	200	Name of Street, Street	Negative in thirty-five minutes.

¹The hanging drop was not examined during the five minutes previous to the given time, hence it is probable that the table indicates a greater uniformity in this respect than occurred in reality. (See footnote under "Morphology and pleomorphism.")

The normal blood of a control rabbit gave no agglutination at 1:10 in forty-five minutes at 28°.

All of these cultures have been grown upon artificial media for from six to twelve months, with the exception of "Pfeiffer," which, as already stated, has been grown on artificial media for the past nine years. Cholera "Pfeiffer" agglutinates almost immediately at a 1:40 dilution, whereas it takes several minutes to produce complete results with the other cultures at this dilution. This susceptibility is not noticeable at the higher dilutions.

PFEIFFER'S REACTION (PERFORMED IN VITRO AFTER THE METHOD OF BORDET).

A loopful of the sodium chloride suspension of the culture to be tested was mixed with a loopful of the above-mentioned immune rabbit serum; a loopful of this mixture then added to a loopful of normal rabbit serum and the result watched in the hanging drop. All of the cultures, with the exception of "554b," agglutinated, the rods became swollen and globular, and in about three hours at 28° began to break up into granular masses; "554b" agglutinated, the rods became swollen but did not disintegrate. In control drops of immune serum alone the rods agglutinated, but no bacteriolysis occurred. In control drops of normal serum the rods retained their motility for three hours.

VI. THEIR PATHOGENICITY.

GUINEA PIGS.

In order to save guinea pigs for other purposes, only the pathogenicity of culture "579" toward these animals has been tested. At the time of isolation about 2 cubic centimeters of a twenty-four-

hour bouillon culture injected intraperitoneally killed a fair-sized guinea pig within twenty-four hours. Eleven months later, after direct passage through three guinea pigs, one loop ¹ of a twenty-four-hour +1 agar culture, grown at 35°-37°, killed a 482-gram guinea pig in four hours. The peritoneal and thoracic cavities showed intense congestion with sero-sanguineous extravasations. The small intestine was greatly congested (much more so than the large) and filled with a yellowish mucoid fluid containing many desquamated epithelial cells, but, microscopically, no cholera spirilla. The abdominal organs were bound together by a fibrinous exudate. Pure cultures were obtained from the peritoneal cavity on agar plates. There were no organisms in the heart's blood.

PIGEONS (FULL GROWN AND OF ABOUT THE SAME SIZE).

Cultures "579," "Scout," "City moat," and "561" were pathogenic when one loop of a twenty-four-hour —1 agar culture suspended in salt solution was injected deep into the pectoral muscle. One loop of culture "A" failed to kill a pigeon. Five loops (about 30 milligrams) of culture "Pfeiffer" failed to kill. Abstracts of the protocols are as follows:

Pigeon 1.—One loop of "579" deep in left pectoral muscle. Dead in fifty-four hours. Congestion of cutaneous and deep vessels of left side. Cloudy swelling of left pectoral. Intestines congested. Microscopically, many curved rods in left pectoral, none in heart's blood. Many Halteridia and shadow corpuscles in blood. Pure cultures were obtained from the left pectoral muscle and heart's blood (three colonies per loop), which agglutinated with the "579" immune rabbit serum at 1:200 in about twenty minutes.

Pigeon 2.—One loop of "Scout" deep in left pectoral; dead in twenty hours; tissue changes as in Pigeon 1; organisms present in pectoral and heart's blood microscopically; many Halteridia present; pure cultures from pectoral and heart's blood which agglutinated with "579" rabbit serum at 1:200.

Pigeon 3.—One loop of "City moat" deep in left pectoral; dead in thirty-four hours; tissue changes similar to first case; organisms numerous at seat of injection, not found microscopically in heart's blood, which contained numerous Halteridia and many shadow corpuscles. Pure cul-

¹The same loop was used throughout the following experiments. When it holds just sufficient culture to fill the cavity of the loop and form a rounded surface on each side, its contents weigh 7 milligrams (wet). Allowing 1 milligram for loss during manipulation "one loop" signifies about 6 milligrams of the culture.

tures obtained from pectoral and heart's blood, which agglutinated with "579" rabbit serum at 1:200.

• Pigeon 4.—One loop of "561" deep in left pectoral; dead in forty-four hours; tissue changes as above; curved rods at site of injection and quite a number in the heart's blood; few Halteridia; cultures from pectoral and heart's blood pure and agglutinate with "579" rabbit serum at 1:200.

Pigeon 5.—One loop of "A" deep in left pectoral; alive and well on tenth day; blood from foot shows very few Halteridia.

Pigeon 6.—One loop of "Pfeiffer" deep in left pectoral; alive and well on tenth day. No Halteridia found in blood from foot.

Pigeon 7.—Two loops of "Pfeiffer" deep in left pectoral; alive and well on sixth day. No Halteridia found in blood from foot.

Pigeon 8.—Five loops of "Pfeiffer" deep in left pectoral; alive and well on fourth day; blood from foot shows a number of Halteridia.

The dose injected into these pigeons seems to be rather large, but was adopted on account of the age of the cultures. I have not been able to test the relative resistance of a pigeon showing marked Halteridium infection on the one hand and one free from it on the other hand, on account of the difficulty of obtaining uninfected pigeons. Culture "Pfeiffer" has been grown on artificial media for such a great length of time that it could hardly be expected to be pathogenic except in large doses. (See footnote under "Morphology and pleomorphism.")

Monkeys.—Several attempts to infect monkeys by feeding have been performed with negative results, but I have notes on one case only.

An old adult male monkey ($Macacus\ cynamolgus$) received the contents of a recent agar slant culture of "579" suspended in -1 bouillon. This was injected by means of a catheter into the stomach. He remained perfectly well for twenty-four hours. During the next four days 35 cubic centimeters of native spirits, called "arac" (containing about 40 per cent alcohol), were injected into his stomach. During part of the time he appeared to be intoxicated and refused to eat. Five cubic centimeters of 1 per cent sodium carbonate was injected into his stomach, followed by the contents of three +1 agar slants of "579" suspended in -1 bouillon. He did not vomit; faeces were normal, and he remained well during a week's observation. The culture "579" had been grown on artificial media for nine months without passage through an animal.

VII. THEIR MORPHOLOGY AND PLEOMORPHISM.

Each of these cultures shows that tendency toward pleomorphism, which is quite as marked as that seen in cultures of *B. pestis* or *B. mallei*, and which is so confusing to the beginner.

It is generally admitted that no two separate lots of media, which are identical in composition and reaction, can be prepared; and though the methods recommended by the American Committee, and the somewhat modified ones employed in these experiments give

comparable results so far as the reaction may indicate uniformity in composition, some bacteria will point out variations not detectable in other ways. I have not been able to reproduce exactly the same type of morphology in any two successive cultivations of the same culture, even on agar from the same batch, although precautions were taken to grow the cultures under apparently identical conditions, to make the preparations from corresponding portions of the growth, and to subject them to, as nearly as could be judged, like conditions of heating, staining, etc. On agar made on separate occasions the variation is still more marked.

In the case of the cholera spirillum which reaches its maximum growth on moist agar in such a short time, variations in morphology will be shown in preparations from the same culture. Thus, one made from the *edge* of a streak on an agar slant, where the younger forms are still multiplying, may present an entirely different appearance from one made from the *center* of the streak, where the older forms have lengthened out and are undergoing involution changes, as shown in figs. 7 and 8.

The variation which is so striking in this instance is not always so apparent in any of the cultures under consideration, nor is it appreciable in a twenty-four-hour culture of the less pleomorphic *B. coli*. Still this difference in the morphology of the younger and older forms of the cholera spirillum must be taken into consideration in comparing the morphology of different cultures, and when it is taken into account the variation on agar from the same batch may not be so marked.¹ (Compare figs. 8, 9, 10, 11, 12.)

It is hardly necessary to say that no permanent variations in morphology were produced in any culture. Figure 5 represents one which was kept in bouillon for ten months. This culture has been described under "Pellicle production in fluid cultures" as "579A." It will be noted that the organisms are somewhat larger than those pictured in figs. 2, 3, and 4. This is but a temporary modification, transmitted while the culture is kept in bouillon, but

¹ It seems worthy of note that in the preparation of the saline emulsions, for the agglutination and animal inoculation experiments heretofore cited, no such precautions were taken. It seems that the failure to do so may account for some of the variations observed. It is not at all impossible, for example, that a "loop" of young healthy cholera spirilla, taken from the edge of the growth on an agar slant, would exert a greater pathogenic action than one taken from the center where the growth is composed of old semidegenerate individuals.

very soon reverting to the shorter, thinner type when grown in peptone solution or transplanted upon +1 agar.

Figure 6 shows some of the long straight and spiral threads which grow out in the pellicle which forms on a liquefied gelatin culture. These are undoubtedly involution forms, for when a portion of such a pellicle is transplanted upon +1 agar, the short curved forms ordinarily met with upon agar develop abundantly. Further, in stained preparations from such an agar surface these threads and spirals take the stain poorly, and after two or three transplants disappear entirely.

VIII. SUMMARY AND CONCLUSION.

- (1) The substance of this article consists, essentially, in making a careful preliminary study of the variations which occur in one culture of the cholera spirillum, and then comparing it with cultures from different sources.
- (2) Certain reasons are given for adopting a modification of the methods of neutralizing media recommended by the American Committee—the hydrogen ion being left out on account of its toxic action.
- (3) The cholera spirillum is not a nitrifying organism, and the successful demonstration of the "cholera-red reaction" in a solution of Witte's "peptone" depends upon the presence of a trace of nitrates. Certain reasons are given for presuming that a variation in the nitrate content of media exists.
- (4) The type of liquefaction produced in gelatin is influenced to a marked degree by the reaction and melting point of the gelatin. Sodium carbonate does not exert a more favorable influence on the proteolytic activity of the cholera spirillum than sodium hydroxide—at least so far as the liquefaction of gelatin is concerned. The proteolytic activity of a culture could not be increased by passage through a series of gelatin tubes.
- (3) The optimum condition for growth is furnished by an albuminous medium containing between 1/50 and 1/100 of a gram-molecule of NaOH or Na₂CO₃ per liter, and this corresponds fairly well with the optimum conditions for the tryptic digestion of fibrin.
- (6) Alkali, detectable by titration with phenolphthalein, is not produced in sugar-free bouillon devoid of sodium chloride.
- (7) Growth in the presence of carbohydrates reveals that the acids produced from glucose, maltose, and saccharose rapidly kill

the cholera spirillum, while those produced from lactose and starch are not toxic—at least within a given time.

- (8) The cultures studied are specifically the same as shown by the Gruber-Durham and Pfeiffer reactions. In order to obtain comparable results, quantitative variations between the agglutinin and agglutinable substance were excluded as far as possible.
- (9) The pathogenicity for guinea pigs, pigeons, and monkeys is mentioned.
- (10) Upon comparing the morphology of the different cultures it was noted that if precautions be taken to make preparations from corresponding portions of the growths, the variations were not so marked.

In conclusion, I wish to express my gratitude to Dr. Paul C. Freer, Superintendent of Government Laboratories, for many valuable corrections of the chemical concepts put forth in this paper and for many helpful suggestions in its editing.

DESCRIPTION OF PHOTOGRAPHS AND PHOTOMICROGRAPHS.¹

- Fig. 1. Showing the variation in the amount and type of liquefaction produced in forty-eight hours in gelatin of varying reaction and dryness. The +1.5 tube has not photographed well; it showed no liquefaction along the stab and but a small, circular liquefied depression at the surface.
 - 2. Coverslip preparation from the human ileum showing the morphology of the organisms of culture "579" as seen scattered about among the desquamated epithelial cells. (The photograph has been carefully retouched by the photographer in order to aid reproduction.)
 - 3. Culture "579," preparation from the edge of a +1 agar slant. grown for twenty-two hours at $35^{\circ}-37^{\circ}$.
 - 4. Culture "579," from just below the pellicle on + 1 bouillon; twenty-four hours at $35^{\circ}-37^{\circ}$.
 - 5. Culture "579A," from the pellicle on +1 bouillon; twenty-four hours at $18^{\circ}-25^{\circ}$.
 - 6. Culture "579," from the pellicle formed on +1 gelatin; five days at $18^{\circ}-25^{\circ}$.
 - 7. Culture "Scout," from the *edge* of an eighteen-hour culture on +1 agar; at $35^{\circ}-37^{\circ}$.
 - 8. Culture "Scout," from the *center* of the same growth from which fig. 7 was prepared; showing older and involution forms.
 - 9. Culture " 561," from the central portion of a twenty-hour culture on + 1 agar; at 35°–37°.
 - 10. Culture "A," from the *central* portion of a twenty-hour culture on + 1 agar; at $35^{\circ}-37^{\circ}$.
 - 11. Culture "city moat," from the central portion of a twenty-hour culture on +1 agar; at $35^{\circ}-37^{\circ}$.
 - 12. Culture "Pfeiffer," from the *central* portion of a twenty-hour culture on +1 agar; at $35^{\circ}-37^{\circ}$.

¹ Taken by Charles Martin, photographer, Bureau of Government Laboratories. (X 880.)



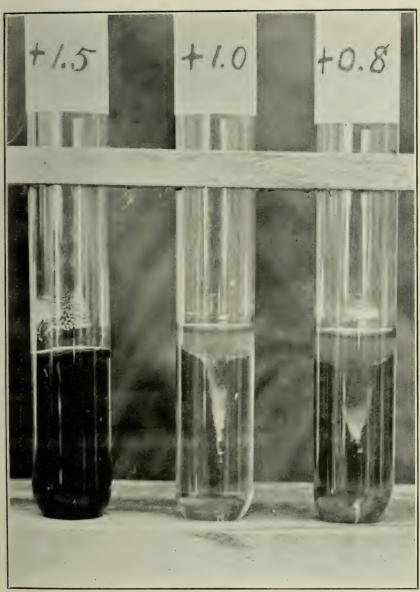
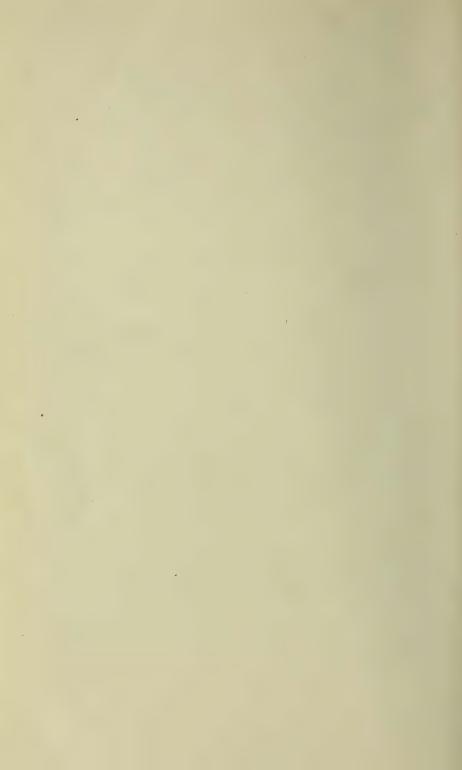


Photo by Martin.

Fig. 1.



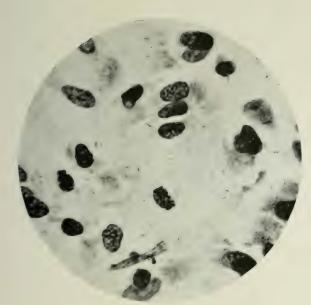
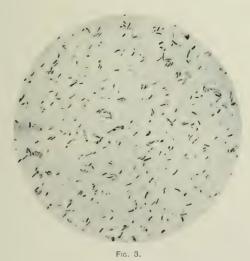
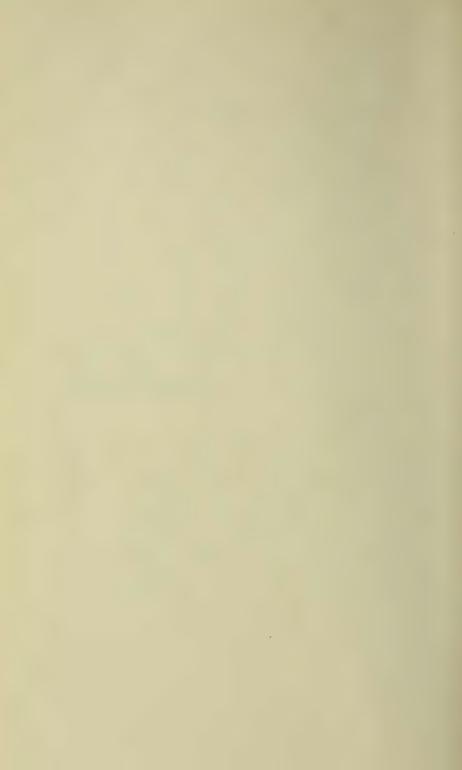


Fig. 2.





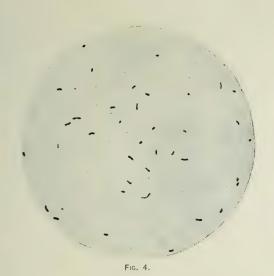


Fig. 5.

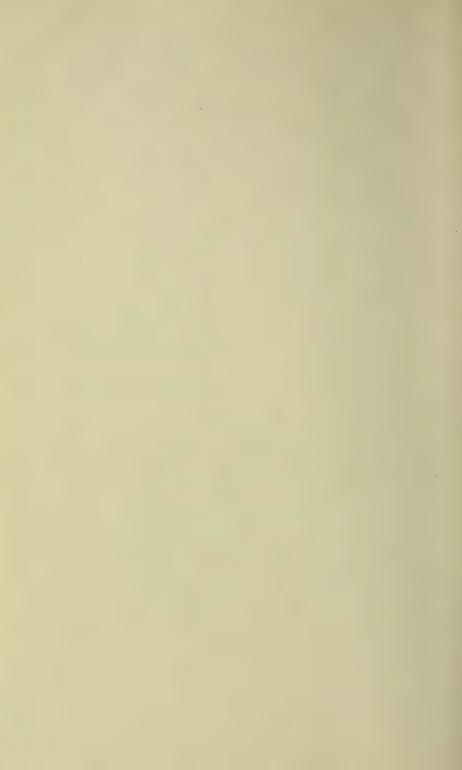




Fig. 6.

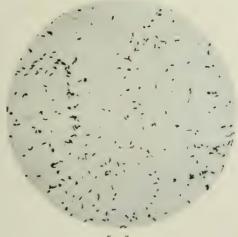
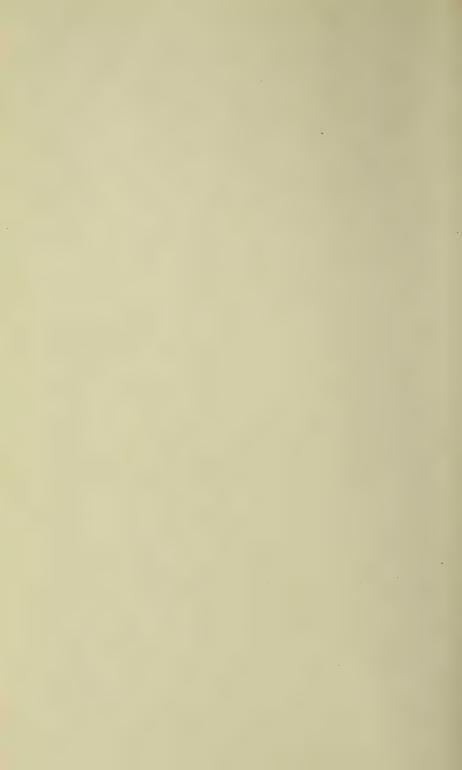


Fig. 7





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Fig. 9.

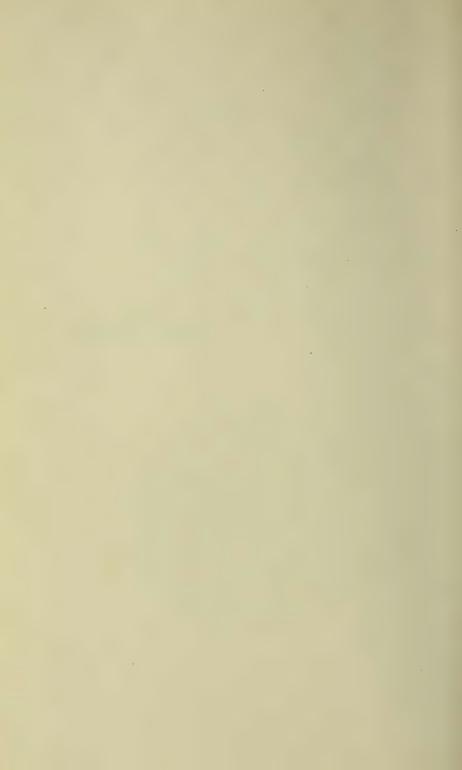
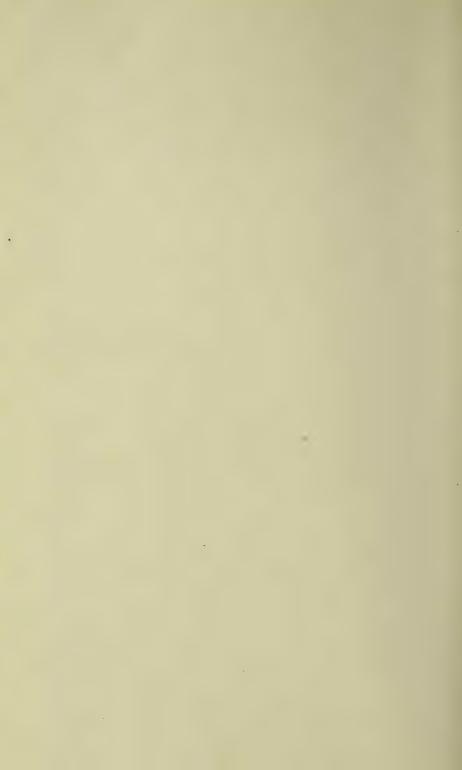




Fig. 10.



Fig. 11.



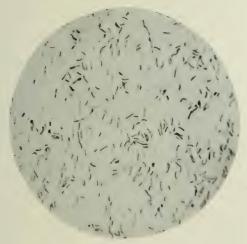


Fig. 12.



No. 20.-OCTOBER, 1904

DEPARTMENT OF THE INTERIOR BUREAU OF GOVERNMENT LABORATORIES

BIOLOGICAL LABORATORY

I. DOES LATENT OR DORMANT PLAGUE EXIST WHERE THE DISEASE IS ENDEMIC

By Maximilian Herzog, M. D., and Charles B. Hare

SERUM LABORATORY

II. BRONCHO-PNEUMONIA OF CATTLE: ITS ASSOCIATION WITH B. BOVISEPTICUS

By Paul G. Woolley, M. D., and Walter Sorrell, D. V. S.

III. REPORT ON PINTO (PAÑO BLANCO) By Paul G. Woolley, M. D.

CHEMICAL LABORATORY

IV. NOTES ON ANALYSIS OF THE WATER FROM THE MANILA WATER SUPPLY

By Charles L. Bliss

SERUM LABORATORY

V. FRAMBŒSIA: ITS OCCURRENCE IN NATIVES OF THE PHILIPPINE ISLANDS.

By Paul G. Woolley, M. D.

MANILA BUREAU OF PUBLIC PRINTING 1904



LETTERS OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., September 30, 1904.

SIR: I have the honor to transmit herewith, for publication in one bulletin of the Bureau of Government Laboratories, the following: I. Does Latent or Dormant Plague Exist Where the Disease is Endemic? II. Broncho-Pneumonia of Cattle: Its Association with B. bovisepticus. III. Pinto (Paño Blanco). IV. Notes on Analysis of the Water from the Manila Water Supply. V. Frambæsia: Its Occurrence in Natives of the Philippine Islands.

I am, very respectfully,

Paul C. Freer,
Superintendent of Government Laboratories.

Hon. DEAN C. WORCESTER,

Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY, OFFICE OF THE DIRECTOR,
Manila, P. I., July 15, 1904.

SIR: I have the honor to transmit herewith and to recommend for publication a paper entitled "Does Latent or Dormant Plague Exist Where the Disease is Endemic?" by Dr. Maximilian Herzog, Pathologist Biological Laboratory, and Mr. Chas. B. Hare, Assistant Bacteriologist.

Very respectfully,

RICHARD P. STRONG, Director Biological Laboratory.

Dr. PAUL C. FREER,

Superintendent Government Laboratories, Manila, P. I.



PART I.

DOES LATENT OR DORMANT PLAGUE EXIST WHERE THE DISEASE IS ENDEMIC?

By Maximilian Herzog, M. D., Pathologist Biological Laboratory, and Chas. B. Hare, Assistant Bacteriologist.

On August 21, 1903, Mr. Henry A. Blake, (1) governor of Hongkong, addressed a communication to the secretary of state for the colonies, entitled "Bubonic Plague in Hongkong: Memorandum by His Excellency the Governor, on the Result of the Treatment of Patients in Their own Houses and in Local Hospitals during the Epidemic of 1903." The writer of the memorandum makes some very startling assertions as to the danger of the spread of plague by animals of the most varied kind, and also comes to the amazing conclusion that there existed in Hongkong during the period of time intervening between June 23 and July 10, 1903, between 8,000 and 9,000 or more individuals among the native population in which plague bacilli were present in the circulating blood in spite of the absence of all clinical symptoms of the disease. The governor calls this condition "latent plague" and considers it a potent factor in the spread of the infection, and a factor which can not of course be reached by the ordinary methods employed to limit the spread of and possibly to suppress plague.

Fully to understand the statements of the governor of Hongkong, it will be well to quote a few paragraphs from his memorandum, which read as follows:

We have from Professor Simpson's report evidence that pigs, calves, sheep, monkeys, geese, ducks, turkeys, hens, pigeons, and rats are susceptible to plague, which may be contracted by food or by inoculation direct,

or by means of suctorial insects. To this list the examination mentioned above adds bugs, spiders, flies, and cockroaches. I may add that quails kept in the market for sale were also found to be infected. In paragraph 22, page 100, Professor Simpson points out that domestic animals suffer from chronic plague, and surmises that this is probably one of the bridges by which the interval of the attacks in man is connected. have for a considerable time been of the opinion that man himself is subject to chronic plague, which may either pass away after a considerable time or continue dormant over the winter months, regaining activity with the annual movement of spring, when the curve of the epidemic is almost constant. This opinion was strengthened by the fact that in August, 1899, the body of a Chinese lift man at Queen's buildings who was accidently killed when attempting to enter the lift while in motion was found to contain plague bacilli. A similar result followed the examination of a man who on the 4th of March, 1901, was killed at Tal Koo Sugar Works by a bag of sugar falling on his head from a height of 20 feet; while on the 2d of April, 1903, in the body of the chief steward of a ship lying in the dock, found floating with a large wound on the head, were also found plague bacilli. Early in June several men from H. M. S. Ocean were sent to the naval hospital, suffering from pneumonia; on examination of their blood seven were found to be suffering from mild cases of plague. In like manner two officers of the Sherwood Foresters who developed feverish symptoms were, on having their blood examined, found to be similarly affected. In the "Boletin Official" of Macao, containing the report on the plague epidemic, 1895, Dr. Gomes de Silva, the medical officer who published the report in 1895, stated that during the height of the epidemic he had discovered plague bacilli in his own excreta.

- (21) In June I directed Inspector Gidley to obtain as many specimens of blood as possible, on slides secured from the Government bacteriologist. He obtained 110 specimens from men, women, and children taken at random. These slides were sent to Dr. Hunter for examination, who reported that in five slides he found plague bacilli, and in seven slides bacilli were present in considerable numbers, some of which showed bipolar staining. They were not sufficiently distinctive, however, to be regarded as *B. pestis*. These slides were obtained between the 23d of June and 10th of July. Since they were obtained there were but three cases of plague in the district, from none of which a specimen of blood was taken.
- (22) I am not unmindful of the fact that these reports were the result of microscopic examination only. But the examination was the same as that on which a great many of the cases treated in the Kennedy Town Hospital were sent to that institution where their cases ran the usual course of plague invasion.
- (23) Now, putting aside the seven doubtful slides, it will be seen that of those people examined at random 4.54 per cent were found to be infected with plague though to all appearances perfectly healthy. If we exclude all the well-to-do, and take the working coolie population alone, they

probably number 180,000, and, assuming the same average amount of infection, there are among that class alone 8,172 persons at present infected in Hongkong. If even a quarter of that average be accepted for the 105,000 inhabitants of the superior class the number of infected will be increased to 9,634. In Appendix G1 will be found the number of rats examined in each month of the present year with the proportion of the infected rats. I am afraid that the incidents mentioned in paragraph 5 weakens deduction as regards Hongkong. But, from whatever source the rats were procured, the proportion of infection in June was 9 per cent or 4.46 per cent more than the percentage of the slides examined, or, if doubtful cases mentioned by Dr. Hunter be included, 1 per cent less, while in January the proportion falls to 0.8 per cent. This being so, with the complete circle of vermin, insects, food, rats, domestic animals, and man all infected in possibly similar-possibly different-proportion, it appears to me unsound to concentrate attention upon the rat as the principal means of bridging over the dormant season.

It appears that Governor Blake, after writing the above, felt the great danger of coming forward with so sweeping an assertion, and in the introduction to his memorandum he himself makes an appeal for a more through scientific investigation of the hypothesis of the existence of latent or dormant plague among the natives of countries where this disease is endemic. He says:

My hypothesis in paragraph 23 may not bear the light of scientific investigation, and, as the hypothesis of a layman, may not carry much weight, but I venture to submit that it is worthy of scientific inquiry, for while a timely glass of water may prevent a great conflagration, and plague at its first introduction may be stamped out by immediate segregation and thorough disinfection, its endemicity once established this is no longer practicable, and, if the hypothesis of dormant or chronic plague in man be ultimately proved to be correct, it is difficult to see how quarantine for even ten days can prevent its annual recurrence, or how any practicable examination of departing passengers can prevent its export from the plague center and possible dissemination elsewhere if suitable conditions for its propagation be present. What the remedy or what the necessary precaution, I leave it for scientific men to determine, but if my hypothesis results in a wider radius of investigation the experiment will not have been useless.

THE RESULT OF BLOOD EXAMINATIONS IN CASES OF PLAGUE.

It is, of course, obvious to any one versed in examinations of this nature that a diagnosis of plague can not be made by a microscopic examination of the blood. Such an examination may possibly be

¹ Omitted in this bulletin.

resorted to in urgent situations, when a rapid clinical diagnosis is desired, but to base far-reaching deductions upon such a microscopic examination is certainly not permissible. What is necessary in order to determine beyond doubt the presence of plague bacilli in the circulating blood is the examination of the latter by cultural methods. We have undertaken a series of such examinations in order to determine whether or not there exists such a thing as latent or dormant plague, as suggested by the governor of Hongkong. Before giving the details of these experiments it will be well to make a survey of the work that has been done with reference to the presence of plague bacilli in the blood in undoubted cases of this disease.

The German Plague Commission (2), in its report published in 1899, page 265, made blood examinations in the case of 141 plague patients, including 17 who were in the period of convalescence varying between the seventeenth and twenty-fifth day after the disease. These examinations were made in the following manner: A drop of blood was obtained from the finger, with the usual asceptic precautions, and inoculated into agar tubes, while at the same time a cover-glass preparation was also made. It was found that in a number of cases where the culture method furnished positive results, the mere microscopic examination failed to demonstrate the presence of the bacilli. Of 124 patients whose blood was obtained during the climax of the disease, in 81 even repeated examinations did not demonstrate the presence of plague bacilli, in 10 the bacilli were encountered only once, while in 33 they were repeatedly found in the blood. Of 81 patients the examinations of whose blood were always negative, 52 recovered and 29 died. Of 10 cases, in which there was a positive result only once, the other examinations being negative, 8 died and 2 recovered. It is interesting to note that in one the bacilli could be detected two and three days before death, while twelve hours before the crisis and at the post-mortem examination it was impossible to find them. convalescent patients invariably failed to show any bacilli in the circulating blood.

Zobolotny (3), in his researches on plague, says that the bacilli are found in large numbers in the blood of animals sick with the disease, but that in the case of human beings the bacteria are much less abundant and sometimes can not be found at all.

Musehold (4), quoting the work of Albrecht and Gohn, reports that the latter examined 122 cases of undoubted plague. In 55 the bacilli were found by the culture method in the circulating blood during life. Four of these 55 cases recovered, and in 2 of them the bacilli were present in large numbers in the circulating blood. In the case of the 51 patients who died the bacilli were found in considerable numbers in the blood on the day of death as well as on the previous one.

Cairns (5) studied the blood of patients during an epidemic of plague occurring in Glasgow in 1900. He gives the results of examinations made inter vitam on cases which subsequently terminated fatally. Four of these may be cited in connection with our investigation. In three fluid drawn from the buboes developed pure cultures of Bacillus pestis, and only in one of the four fatal cases was it possible to obtain cultures of the bacilli from the blood during life. The other three cases gave negative results. In one of these, seven daily consecutive examinations were made, as well as one shortly before death, all of them proving negative.

Calvert (6) studied two epidemics of plague in Manila in 1900 and 1901. He found that the clinical examination of the blood for Bacillus pestis gave unreliable results, it being impossible to place any weight on the negative findings. He made his examinations by taking the blood from the ears of the patients at intervals of four hours, and examining it in smears as well as by the culture method. This plan was followed until the death or recovery took place. Thirty-six cases, 4 of which recovered, were examined in this manner, most of them being followed to autopsy, when the plague organism was demonstrated by culture and even by animal inoculation.

This author gives the following table of positive findings of the bacilli in the blood:

	Per cent.
24 hours before death in 31 cases	100
48 hours before death in 7 cases	. 22
72 hours before death in 5 cases	16.12
96 hours before death in 2 cases	6.45
120 hours before death in 1 case	3.22

On looking over the table it appears that the plague bacilli could be found in all fatal cases twenty-four hours before death, but that forty-eight hours before death the percentage of positive findings was much smaller. In searching for the bacillus in the blood of plague patients who finally succumb to the disease, the chances of finding the microbe five days before the fatal termination are only one out of thirty. All of this shows that even in fatal cases the plague bacilli are not found in the peripheral blood at an early date.

Jennings (7), in his Manual of Plague, page 112, says that it is extremely rare to find plague bacilli in the blood in large numbers except immediately before death. Their absence, therefore, in the early stages of an attack is frequent, and must not be regarded as a negative diagnosis of plague.

Terni (8), who studied the plague in Rio de Janeiro, in an excellent article on the disease makes the following statement:

"The examination of the blood is by no means reliable. It is astonishing that Galeotti places any value in blood examinations in the diagnosis of early plague."

Terni found that in many cases which were diagnosed as plague septicemia, examinations of the blood, both microscopic and by culture method, were negative. This was true even at the post-mortem examination, because the bacilli were exclusively localized in the lymph channels. Even in the most profound cases, in individuals particularly predisposed to the disease, the bacilli were found in the blood only in moderate numbers. Their presence could never be compared with what has been found to be true in connection with other septicemic microbes, such as anthrax or diplococci. A multiplication of the plague bacilli is found only in exceptional cases in the circulating blood during the agonal stage.

One of us has been studying for some time the morbid anatomy and histo-pathology of a number of cases of plague. These studies appear fully to confirm the statement of Terni to the effect that plague, as a rule, is not to be looked upon as a true septicemia, but, on the contrary, as an infection particularly confined to the lymphatic system. Even in cases where sections from the lymph glands contain innumerable bacilli, the lumina of the blood vessels are generally free from such microbes. In fact, in the study of sections from all of the internal organs when plague bacilli are seen they are always found in the lymph channels or lymph spaces and not in the blood vessels.

Powell (9) has recently reported the result of 3,400 blood examinations of febrile diseases in Bombay. Most of these cases were malaria, but 117 of them were plague. In only 15 of the latter were the bacilli easily seen in blood smears. With reference to the finding of the bacilli in the blood in cases of plague, the author says: "As regards the recognition of the plague bacilli in the finger blood, for some years I was very sceptical about the reports of certain medical men, and until within the past eighteen months had been unable to detect the bacilli except on cultures. At the beginning of this year there was a particular type of septicæmic plague, in which the bacilli were found in every case. Such cases in my experience always died. One case seemed to be convalescent and had a normal temperature for three days, but suddenly died. Plague bacilli were found eleven days before death."

The *Indian Plague Commission*, speaking of the bacteriologic diagnosis of plague by microscopic examination of suspected material, makes the following statements:

(156) In the case of blood derived from a patient who is suspected of suffering from plague, the detection of bacteria possessing the morphological characters of the plague bacillus will (especially if these are present in large numbers and when it is determined that these become decolorized by Gram's process) be conclusive evidence that the patient is suffering

from plague. The mere finding of a few isolated bacteria arranged together as diplococcal forms can not, especially when Gram's test has not been applied, be accepted as establishing the diagnosis of plague. We have in view here in particular certain suspected cases which occurred in Calcutta in 1896, in which it was claimed by Professor Simpson that the diagnosis of plague was confirmed by his bacteriological examinations. We would note with regard to these first that in some of the suspected patients only a few isolated diplococcal forms were found after long searching through a number of films prepared from the blood. Again, in view of some of the figures reproduced in the record of Dr. Simpson's evidence, particularly Figs. I, VI, C, and F, it is important to note that it does not seem to have been determined whether bacteria became decolorized when treated by Gram's process, and, lastly the fact must not be lost sight of that saprophytic diplococci of various kinds are widely distributed in nature, and that there is always a possibility of microscopical preparations containing as contaminations a small number of such diplococcal forms.

In looking over these statements (all that we can find in the literature at our disposal), one is certainly impressed with the fact that the finding of plague bacilli in confirmed cases of the disease, except very shortly before death or in the rarer cases of plague septicæmia, is the exception and not the rule. Indeed, we should be mindful of the fact that plague, as a rule, is not a septicæmia but a bacterial infection localized in the lymphatic system. It is, therefore, from a purely theoretical standpoint, highly improbable that there should exist a dormant, latent clinical form of the disease in which the patients harbor the bacilli in the circulating blood.

Our investigations to determine whether such is the case or not were made on a number of native Filipinos and Chinese of this city. We attempted to get material which, if latent plague exists at all, would give us some evidence to this effect, therefore we selected natives from houses in which plague cases had occurred. We examined a number of the inmates of Bilibid Prison, particularly such as were under the most unfavorable hygienic conditions, namely, insane prisoners and those of the third class who were most crowded in their quarters. Since we could not always get natives of this description, we selected also a number which were not under particularly unfavorable hygienic conditions, such as native police officers and native members of the Constabulary.

While plague has never at any time been as widespread here as in Hongkong, a sufficient number of cases have occurred to make it clear that the disease is endemic, though fortunately not markedly epidemic.

According to the monthly sanitary reports of the Board of Health plague has prevailed in Manila since 1900 to the following extent:

Prevalence of plague in Manila, 1900 to 1904.

1900.

Month.	Chinese.	Filipi- nos.	Americans and other Caucasians.	Total number of cases.	Total number of deaths.
January February March April May June July August September October November December	52 43 13 14 5 8 6 5	15 12 12 11 7 5 8 9	2	18 48 64 54 22 19 13 18 6 7 7	11 35 48 44 18 11 7 11 9 5
Total	186	82	3	271	199

1901.

January	4	3		7	
February	15	11	1	27	2
March	49	14		63	5:
April	73	38		111	9:
May	97	40		137	12
June	24	30	1	55	5
July	18	20	1	39	3
August	12	16	1	29	2
September	7	4		11	1
October					
November					
December	1	4	1	6	
Total	300	180	5	485	42

1902.

January	
February 1	1
March 1	1
April May	
June 1	1
July	
August111	1
October 2	2
November 1 1	1
December 2 2	2
Total	10

Prevalence of plague in Manila, 1900 to 1904—Continued.

1903.

Month.	Chinese.	Filipi- nos.	Americans and other Caucasians.	Total number of cases.	Total number of deaths.
January February March April May June July August September October November December	7 18 35 16 9 3 10 3 3	1 10 15 15 15 9 23 11 1 1 2 2	2 2	1 17 33 52 27 32 14 11 4 3 2 2	1 15 33 49 23 25 9 9 4 2
Total	104	90	4	198	174

1904.

January February March April May	3 8 9	6 6 12 7 8	1	10 7 15 15 17	7 6 14 15 16
Total for five months	24	39	1	64	58

SUMMARY.

Reported in—	Plague cases reported.	Plague cases fatal.
1900	271	199
1901	485	427
1902	10	10
1903	198	174
1904 (Jan. 1 to May 31)	64	58

It appears from these statistics that plague had completely died out during four of the months of 1902, since during this period not a single case came under observation. However, since August, 1902, until the time of writing the present report there has not been a month entirely free from plague, though the figures have generally been low, the maximum during this period being reached in April, 1903, when 52 cases of the disease were reported.

The object in giving these figures in connection with our report is to show that plague has been sufficiently prevalent here for several years, so that blood examinations should furnish evidence of latent plague provided that such a form of the disease exists at all.

The figures of plague morbidity for Hongkong during the same years are as follows:

1900	Cases.
1001	1,086
1901	1,637
1902	540
1903	1,135

BLOOD EXAMINATIONS OF 245 APPARENTLY HEALTHY NATIVE FILIPINOS AND CHINESE.

The method employed to ascertain whether apparently healthy Filipinos or Chinese of Manila have any plague bacilli in their blood was as follows:

The bend of the elbow, generally of the left arm, was very thoroughly cleansed first with strong alcohol and then with a strong solution of mercury bichloride, and finally with alcohol and sterile distilled water. A rubber bandage was then placed around the arm above the elbow and 1 cubic centimeter of blood was drawn from one of the veins by the aid of a sterile hypodermic syringe. The blood so obtained was added to 50 cubic centimeters of bouillon in a flask. The bouillon used was prepared as usual, and, when neutral to litmus, 0.5 gram of bicarbonate of soda was added to each 1,000 cubic centimeters of the bouillon, making it slightly, but decidedly, alkaline. This forms a very excellent culture medium for plague bacilli. As a control experiment in some of the cases about twice the amount of blood was drawn from the vein, and the 2 or 3 cubic centimeters so obtained was distributed in two flasks. One of the latter was then immediately inoculated with a very small amount of material from a young plague culture. This was, of course, done to see whether plague bacilli, if present, would develop in the bouillon in the presence of a small amount of freshly drawn blood. It may be said that the control flasks always developed a typical plague growth, so that evidently nothing in the arrangement of the experiment prevented development of the plague bacilli if any were present. The culture flasks to which blood had been added were kept either in a dark chest at room temperature or in an incubator which was fairly constant at 35° C. The media were inspected daily and when a growth developed it was examined in stained preparations and by culture on agar.

The native Filipinos whose blood was examined included 32 laborers from the Serum Institute and the morgue. The native servants of the latter, where all the plague post-mortem examinations of the city are made, are, of course, particularly exposed to infection, and would be especially prone to show latent plague provided that such a condition exists. These 32 cases were kindly examined for us by Dr. E. H. Ruediger, bacteriologist in the Serum Institute, whose technique differed from the method generally used only in that he employed a 5-per-cent carbolic acid solution to sterilize the elbow. Dr. Ruediger also examined all of his 32 flasks by stained cover-slip preparations and by culture methods whether they showed any change in appearance or not.

The blood examinations in all of the 245 cases were made between March 4 and May 20, 1904—i. e., during a period when from thirty-five to forty cases of plague were reported in Manila.

The following is a summary of the examinations:

On March 4 there were examined 5 native Filipinos from a house in Santa Cruz in which an ambulatory case of plague terminating in embolism of the pulmonary artery had occurred. Specimens were taken from 10 native Filipino police officers on April 6 and 7. Nine of these men lived in infected districts-i. e., those in which cases of plague had recently occurred; I came from a noninfected district. Ninety native Filipinos, members of the Philippines Constabulary, were examined between April 13 and 30. These men live in barracks, but are often free to visit their families and friends. During the first week in May 32 native Filipinos, laborers at the Serum Institute and the morgue, were the next subjects investigated. In the former place the various vaccines and sera, including plague vaccine and serum, are prepared, and in the latter are performed all the necropsies on plague cases. Fifty-eight native Filipinos, prisoners in Bilibid Prison, were taken on May 12 and 13. In this place about 4,500 men are confined. Last year a number of the inmates died from pneumonic plague, but during this one no case has occurred there, although one of the prisoners died of pulmonary plague four days after his discharge. Of these 58 men 16 were insane prisoners, all in advanced stages of degenerative mental disease, and the remainder were the so-called third-class prisoners, who lived under the most unfavorable conditions to be found in the prison.

On May 16 to 20 there were examined 50 Chinese, small shopkeepers, clerks, and coolies, either from houses in which plague cases (in one instance two such cases) had occurred or from those adjoining.

RESULTS OF THE EXAMINATION.

Most of the flasks to which 1 cubic centimeter of blood had been added remained sterile; although a few developed growths which, however, were clearly contaminations from the air, such as common molds or similar forms of life. One of the cases taken by Dr. Ruediger developed Staphylococcus pyogenes aureus; and those of two other natives developed a bacillus which, when examined in a stained cover-glass preparation, might possibly be mistaken for Bacillus pestis. One of these organisms, however, in culture looked very different from the bacillus of plague and also retained Gram's stain. This bacillus developed in a flask to which had been added blood from one of the insane prisoners. The other growth occurred in a flask containing blood from a member of the Constabulary. This organism also greatly resembled morphologically the plague bacillus, but it kept Gram's stain, and when rubbed in large amounts on the shaved abdomen of a guinea pig failed to produce disease. In short, not in a single instance out of 245 examinations of persons of whom a large percentage had certainly been greatly exposed to plague infection did we find any evidence of the existence of plague bacilli in the blood.

CONCLUDING REMARKS.

From our investigations conducted on 245 native Filipinos and Chinese it may be safely concluded that a condition of latent or dormant plague does not exist in Manila, and there is hardly any reasonable doubt that it does not exist in Hongkong. There certainly has not been furnished the slightest proof of such a character as to stand the searchlight of exact methods of bacterial investigation to indicate that there is such a thing as latent human plague, with the presence of plague bacilli in the circulating blood, in the absence of clinical symptoms of the disease.

The governor of Hongkong himself, in his memorandum, clearly sets forth some of the circumstances which unite to make it practically impossible to completely eradicate the disease in Victoria City.

In Hongkong (1) (memorandum, par. 3) it is the custom of the Chinese—if they can possibly do so—to dump human corpses dead from plague into the street, in order that they may not be found in the houses and thereby subject the inmates to quarantine, disinfection, etc. In spite of measures to prevent this procedure, the number of corpses dead from the plague and so disposed of has, during the ten years preceding 1898, increased from 25.1 per cent to 32.7 per cent.

The Chinese in Hongkong, according to the governor's memorandum, offer passive resistance to the catching of rats in their houses, being afraid that plague bacilli might be found in the rodents, as this would lead to measures of disinfection or to the repair of their houses. The Chinese rat catchers are said to be dishonest; they fail properly to label the rats, so that infected houses escape detection, and they import rats from the outside of Hongkong and label them at random. In general they are very unreliable in their work and are actuated solely by the desire to secure from anywhere the largest number of rats in order to obtain the premium offered for each.

To those who know how Chinese houses are constructed [says the memorandum, in par. 6], it will be apparent that effective fumigation is practically unattainable. While, even if the spraying process, scrubbing and disinfection of clothing reached externally everything in the room, it would not kill vermin lying deep in the joints and cracks of the tables, chairs, and settees, or beds. Nor would it reach the vermin with which the heads of the poorer classes of coolies are infested. But apart from this, what took place in many cases when a case of plague was detected was that before the constable could arrive to take charge of the house, goods liable to injury by disinfection were removed by the door, or, if too late for this, were taken on to the roof, always easily accessible, and deposited in some neighboring house.

In W. J. Simpson's report (11) "On the Causes and Continuance of the Plague in Hongkong, etc.," which is quoted in the memorandum of the governor, we find the following statements as to the sanitary conditions in the Chinese quarters in the city of Victoria.

When a case of plague has once occurred in a house, there is a great tendency in subsequent years for the same house, or that adjoining, or that on the opposite side, or that close by, to be attacked with plague. When plotted out on a map, the distribution of plague appears to be closely connected with previous infection of the house or of a defined locality, the infection having been retained in an unrecognized form in the interval. The houses which suffer principally are, speaking generally, the most insanitary and the oldest. It has already been mentioned, how closely packed the buildings are in the older portions of the town, narrow streets and high houses being the leading features, by which the admission of sunlight and fresh air is considerably obstructed. Narrow streets and high houses, however, are not peculiar to Hongkong; they are to be found in other towns, with their injurious effects on health, but in Hongkong there is moreover in the Chinese quarters a defect in the construction of the houses which intensifies the obstruction of light. The rooms are long and narrow, with a window at each end, the front window looking into a wide and covered veranda, and the back window into a small open space at the back, which forms a sort of wall between the two houses. The lower floors of many of the houses are remarkable for their darkness, and this in a region not far from the Tropics; they are also frequently damp.

Since the epidemic of 1894 many of the lower floors of the worst kind have been changed into storerooms to contain the goods and merchandise for which Hongkong is in entrepôt. These storerooms as a rule are infested with rats, which at times find their way up to the rooms on the higher floors. The basements are generally rat ridden, both floors and walls, and from the walls being often hollow it is easy for rats to reach the upper floors.

The admission of sunlight into the dwelling rooms of Chinese tenement houses is still further obstructed by the subdivision into several cabins or compartments, sometimes numbering up to six, which every room is subjected to. Each cabin is let out to a separate tenant and not infrequently accommodate a separate family. These compartments or cubicles are windowless rooms, and are often so dark that it is impossible for any one, coming directly from the light outside and drawing or opening the door of the cubicle, to see at once whether it is occupied * * * . Some attempts have been made to improve this state of things by limiting the height of the subdividing walls to six feet. The condition which obtained before this improvement has made it somewhat difficult to realize, for what I am describing is that which now exists. Fresh air and sunlight never get into the cubicles except perhaps the compartment at each end of the room opposite the window. The subdivision of a single room into a number of rooms called cubicles is an ingenious device for crowding together a large number of people into a small space and securing a correspondingly large rental, but it is an arrangement which engenders disease and favors its spread. There is no doubt whatever that every such windowless cubicle is unfit for human habitation and should not be permitted * * *. Probably another cause for the continuance of the plague, besides the insanitary condition of the houses referred to, is the very inadequate number of latrines and urinals with which Hongkong is provided. The number of public latrines appears to be twenty-nine belonging to the Government, and seventeen to private owners. The total number of seats is 1,202. Most of them have urinals attached, and in addition there are three small public urinals in the town. Seeing that all the men and boys go to the public latrines, there are no sanitary appliances in the houses except earthen pots, which are used exclusively by the women and children. The total inadequacy of the latrine accommodation provided is obvious. It is not one seat to one hundred of the male population. On the Kowloon side of the colony the latrine and urinal accommodation is still more deficient. Large blocks of houses have been built, and not a single latrine or urinal provided by the builder of the block. It is impossible under these conditions that the ground should escape being sewage polluted * * * *. The existing latrines are far from being models of what they should be. They are in fact insanitary in structure and deficient in light and ventilation.

Quite recently Surgeon-General Evatt, P. M. Q., His Majesty's troops, Hongkong, has been quoted by the daily papers in an interview in which he speaks in the strongest terms of the insanitary conditions, which he holds responsible for the continued prevalence of plague in Hongkong. He calls Victoria City the plague-distributing center of the world, a standing menace to the human race. So we have the most convincing evidence, both official and unofficial, as to the vicious sanitary conditions in Hongkong. These furnish the soil in which plague thrives, from which it can not be completely eradicated, and from which it breaks forth again and again in menacing epidemic form.

From our own investigation carried on in Manila, we have certainly good reasons to deny the existence of latent or dormant plague in our city. The statements of those who have looked into the conditions in Honkgong likewise appear unfavorable to the theory of the governor, and they offer an explanation more in accord with what we do know positively as to the nature of plague infection, and as to the spread of infectious diseases in general.

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PART II.

BRONCHO-PNEUMONIA OF CATTLE: ITS ASSO-CIATION WITH B. BOVISEPTICUS.

By Paul G. Woolley, M. D., Director of the Serum Laboratory, and Walter Sorrell, D. V. S., Veterinarian Serum Laboratory.

The reason we care to dwell upon these cases is not that the above-mentioned pulmonary affection is uncommon or not well known, but because of its relation, here at least, to hemorrhagic septicæmia.

It is agreed by all authors that the causes of catarrhal pneumonia, in animals as well as in man, are not always the same. in general the chief ones are also those of acute bronchitis. Following this it happens, either because of swelling of the mucous membrane, or by aspiration of bronchial secretion, that the small subdivisions of the bronchi became plugged, and a condition of atelectasis arises in that part of the lung supplied by the affected bronchioles. Then because of the locus minoris resistentia furnished by the atelectatic part, and because of the usual presence of infectious material in the bronchial secretions and in the aspirated material, inflammatory processes occur, which may be limited or which may spread by continuity to the neighboring tissue. The changes following atelectasis may either be a gradual atrophy of the affected parts of the lung, or following infection, bronchiectasis, abscess formation, pleurisy, gangrene, etc., resolution, or calcification. The disease occurs especially in young animals and in older ones whose physical condition is below par. In young animals it may be epizoötic.

The symptoms are said to vary. Schneidemühl says that the

first signs point to acute bronchitis, upon which ensue pulmonary symptoms, such as increased respiratory frequence and cough.

The course of the disease according to Diekerhoff and Schneidemühl is neither typical nor regular. Sometimes it progresses very rapidly. In other cases it may last for weeks or months.

In the more chronic ones a purulent pneumonia with pleuritis and pulmonary gangrene may ensue and death follow. It is in this last group, that of chronic cases, that ours belong.

These occurred in the herd of the Government Serum Laboratory. The calves were imported for use in the preparation of vaccine virus. The cattle were used in the preparation of antirinderpest serum. All of the calves originally came from China, and upon arriving in Manila had been treated with prophylactic doses of antirinderpest serum and kept under careful observation for several weeks before they were used for the intended purpose. At the time of vaccination there was no indication of disease in any member of the herd, their appetites were good, they were in excellent physical condition, and their temperature curves were normal. (Inasmuch as tuberculosis has never been observed in cattle here the vaccine calves are not slaughtered after collecting the virus, but are kept on hand until disposition can be made of them in some other way. As a rule, the physical condition of the animals improves after taking the vaccine. When this is not the case slaughter is resorted to when there is no particular reason, as in the present instances, of allowing the animals to live.)

The steer mentioned below was also imported from China for use in the preparation of antirinderpest serum, but its condition was never satisfactory for this purpose.

The histories follow:

CASE I.

Calf No. 437 was received at the laboratory on January 12, 1904, and received 100 cubic centimeters of antirinderpest serum at once. It remained apparently well with a normal temperature until January 24, when the latter rose to 40.7° C. The next day the temperature was 40.8° in the afternoon, and the following day it reached 41.2°. From this point it gradually declined to normal on January 30. On February 1, 5 cubic centimeters of virulent rinderpest blood was given to the animal, and on the third day after, it was vaccinated. Following this operation, there was what at first appeared to be the usual temperature of vaccinia, which reached 40.6° on the fifth day after vaccination. But this temperature instead of declining persisted with slight variations between 39° C.

and 41° C. until March 1, when it became normal and stayed so until death, March 24, 1904. On several occasions the blood of the animal was examined for trypanosomas with negative results.

During the course of the disease, the animal gradually lost weight in spite of a reasonably good appetite, and as it progressed the appetite became impaired and the coat rough and stairy. There was no cough, nor in fact any other notable symptoms than the gradual wasting.

The autopsy was made about twelve hours after death. The only appreciable lesions present were in the lungs. The other organs were in an apparently healthy condition, with the exception of the prescapular lymph glands, which were enlarged and edematous.

The apices of the lungs were chiefly affected. These portions of the organ were almost completely solidified. The surface of the hepatized portions were mottled with yellowish-white areas which stood out distinctly upon a reddish-purple ground. In palpating, the finger was able to detect that these lighter areas were firmer than the darker colored portions of the lungs, which had an edematous consistency. Over several of the nodules there was a thin, fibrinous membrane under which the pleura was congested and roughened. Upon section, the affected portions of the organs cut with increased resistance, and from the incisions a frothy serum oozed. The general color of the surface was dark red, mottled by the grayish yellow of the sometimes almost caseous nodules. Sections of the latter had all the macroscopic characteristics of the gray hepatized tissue of pneumonia, being granular and dry. In an occasional one the process had gone beyond the simple hepatization and the center had softened, producing a rather creamy material.

From several of such tubercles, cultures were made upon agar and in bouillon, and pieces of the tissue were placed in Zenker's solution, and absolute alcohol, for further study. Smears made from the pulmonary nodules showed a few small ovoid bacilli, which stained with the ordinary anilin dyes and were decolorized both by Gram's and Gabbett's methods.

Cultures of organisms were obtained which had all the characteristics of the bacillus described by one of us as the cause of an epidemic of hemorrhagic septicæmia among the carabaos of the Government corrals during the past year. The chief characteristics of this organism were its polar staining, rounded ends, nonmotility, and occasional encapsulation. It grew invisibly on potato, did not produce gas in either solid or liquid glucose media, did not coagulate or acidify milk, did not form spores or liquefy gelatin, but did reduce nitrates and also gave the indol reaction. It was pathogenic for guinea pigs, causing death within twenty-four hours after intraperitoneal injection of 1 cubic centimeter. It was the Bacillus bovisepticus of Kruse.

The tissues preserved for sectioning were embedded in celloidin, and sections from these were stained with hæmatoxylin and eosin, by Gram's method and by the carbol-fuchsin-acid one for tubercle bacilli. The stained sections showed a generally edematous condition of the parenchyma. The trabeculæ were somewhat thickened and the fibrous tissue of the affected portion of the lungs was generally increased. The epithelial linings of the

bronchi were convoluted and hyperplastic, the cells being in many places three or four layers thick, and in the dilated lumina there were considerable accumulations of epithelial cells, polymorphonuclear leucocytes and a minimal amount of fibrin.

The air spaces of the affected lobes which were not involved in the consolidation were filled with a granular material with which there were occasional desquamated cells and leucocytes. The peri-bronchial tissue was the seat of well marked round cell infiltrations and the peri-bronchial connective tissue was considerably increased.

Sections of the small nodules of consolidation showed that the chief change in these was a coagulation necrosis. The centers of these areas were crowded with leucocytes and granular, cellular detritus enmeshed in fibrin. The walls of the air cells were perceptible as faintly pink-stained bands in which no nuclei or only shadows could be seen. As the periphery of these areas was approached, the lines became more distinct, and about the latter was a zone of congestion. There were no giant cells seen in any sections, and no "acidfast" bacilli could be found. There were, however, a number of very short bacilli between the cellular contents of the abscesses.

CASE II.

Calf No. 423 was received from Hongkong on January 6, 1904, and given a preliminary prophylactic dose of 100 cubic centimeters of antirinderpest serum. It was vaccinated on January 13, after which its
temperature rose to 40° C. where it remained with slight remissions
until January 19, reaching normal on the 20th. On January 21 it received 10 cubic centimeters of virulent rinderpest blood, and following
this the temperature again rose and varied between 39.2° and 40.6° for
the next eight days. On February 1 it received 50 cubic centimeters of
virulent blood, and following this there was again a rise, which, however,
was transient. From this time on the temperature remained within normal
limits. Death occurred April 30, somewhat more than three months after
the arrival of the animal at the laboratory.

During the last month or five weeks of its life a steady and gradual decline was evident. The animal lost weight in spite of a constantly fair appetite. Upon several occasions the blood was examined for trypanosomas, each time in vain. No cause could be found for the wasting. There was no cough, in fact no other symptoms than the gradual emaciation, and increasing weakness.

The day before death the animal was unable to stand but was lying in its stall in the hospital shed, eating the grass before it. It died the following night and was found in the morning in its sleeping position.

Upon opening the thorax a considerable amount of clear serofibrinous fluid gushed out. The peritoneal cavity contained no fluid and was apparently normal. The subpleural and mediastinal tissues were edematous and gelatinous in appearance. The prescapular glands and periglandular tissues were also edematous. The liver appeared pale but otherwise normal. There were a few petechiæ in the capsule of the spleen, which was of normal size and consistence.

The cecum contained a number of oesophagostomas and fasciolas. There were no gastro-intestinal hemorrhages or ulcerations.

The lungs were the seat of a patchy hepatization, which involved about one-half of the entire pulmonary tissue, chiefly the apices and anterior lobes. In the hepatized areas, and perceptible to the eye and finger, upon the surface of the lungs, were small nodules. These were small and paler than the adjacent tissue, which had an almost purple color. Section of these organs showed that the fibrous tissue was generally increased in the affected portions, the trabeculæ being quite prominent. The surfaces of cut sections showed mottled gray and red, the gray being most apparent in the centers of the lobules. Certain of these gray areas were quite dry and almost caseous, and were as large as a navy bean.

From these nodules cultures of a nonmotile polar-staining bacillus which did not stain by Gram's method and did not form spores were obtained on agar. The colonies in agar varied in size from 0.5 to 1 millimeter in diameter, were thick and opaque at their centers, were not chromogenic, had smooth, regular edges and were somewhat sticky and mucilaginous. On agar slants after twenty-four hours the growth was colonial, but with a tendency to confluence. The water of condensation was clouded.

On glycerin-agar the growth was more luxuriant, quite thin but almost opaque, and so moist that it was inclined to run down the surface of the slant.

Milk became just perceptibly acid after seventy-two hours, but without coagulation or reduction of the litmus. The growth on potato was invisible. In glucose broth and agar no gas was formed. In Dunham's peptone, to a liter of which 5 cubic centimeters of a 5-per cent solution of potassium nitrate had been added, a brillant cholera red reaction could be obtained after twenty-four hours. After seventy-two hours a pellicle appeared in the ordinary bouillon; the medium was diffusely clouded and a flocculo-gelatinous sediment was thrown down.

Of a twenty-four-hour-old broth culture, 1 cubic centimeter was injected into the peritoneum of a healthy guinea pig. The animal survived this treatment.

Cellodin sections cut from material fixed in Zenker's solution, and stained with hematoxylin and eosin, showed changes that correspond in all general respects with those described in the previous case.

CASE III.

Calf No. 415 was received at the laboratory on December 29, 1903, and given the usual prophylactic dose of 100 cubic centimeters of antirinderpest serum. The temperature remained normal until after vaccination, which

¹ Specimens of these parasites were sent to Chas. Wardell Stiles for identification.

² This method was suggested to us by Dr. W. B. Wherry. For some time we have all been troubled by the inconsistency of the peptone used in making Dunham's solution. Dr. Wherry, however, found that a constant reaction could be obtained by adding traces of nitrates to that fluid.

was done eight days later. Following this, on the third day, the temperature rose abruptly to 41.6° C. and then gradually fell to normal within the next week. On January 20, 1904, it was given a subcutaneous injection of 10 cubic centimeters of virulent rinderpest blood, following which there was a reaction of 1.4° C., after which the temperature fell to normal and continued so to the time of death on May 14, 1904, almost five months after the animal had been received. At no time were trypanosomas found in the blood. The clinical history in the case was not unlike that in the previous one; the salient points being gradual loss of weight in spite of retention of appetite, normal temperature, no cough, roughened and stairy coat, and loss of strength.

At autopsy the chief lesions, and in fact the only macroscopic ones, were in the lungs. There was not as much edema as in the previous case, and there was a less general pulmonary involvement. But here, as in the other ones, the apices and anterior margin of the lungs were partially solidified, mottled with red and gray, and filled with small, firm nodules, some miliary and some the size of a large bean. Some of these on section proved to contain a semifluid purulent material, while others were dry and gray.

Cultures were made as before from these nodular lesions and in this case two organisms were isolated, one *B. pyocyaneus*, the other a polar staining bacillus agreeing in general with the organism previously described, but varying from it in some cultural characteristics.

Morphologically, it was identical with the organisms from the previous cases. Culturally, variations were most marked in broth and on agar. On the latter, the growth was generally composed of colonies, but these were somewhat larger than the ones described for *B. bovisepticus*. The growth was much more luxuriant and whiter and there was a decided tendency to confluence on the part of the colonies.

In bouillon there was at first a diffuse cloudiness with no sediment and no pellicle. Later, a gelatinous sediment was deposited, a very delicate pellicle was developed, and the body of the medium became clear. There was also a deposition of floccules on the sides of the tubes.

The cholera-red reaction was obtained after twenty-four hours. Milk was unchanged, and there was an invisible growth on potato.

Intraperitoneal injection of 1 cubic centimeter of this organism killed a healthy guinea pig in less than twenty-four hours. At autopsy an acute hemorrhagic peritonitis was discovered, and the organism recovered.

CASE IV.

Calf No. 464 was received at the laboratory on January 12, 1904, and was injected with 100 cubic centimeters of antirinderpest serum. The following day its temperature was 40.1°, and on the succeeding it was normal and remained so for the next ten days. On January 20 it was vaccinated with vacine virus, and following this its temperature rose, reaching a maximum, 41.1°, on the day after the vaccine was collected. Two days later it was again normal. On February 1, 1904, 10 cubic centimeters of virulent rinderpest blood was injected subcutaneously, and

following this the animal's temperature became quite irregular, reaching 40.5°, and with daily remissions of 1 to 2 degrees; but after twelve days the maximum had fallen to about 39.2° C. and with smaller remissions. This continued until death occurred, May 9, 1904, about four months after its arrival in Manila. Up to this time it was able to eat, but it gradually became emaciated and very weak.

At autopsy the lungs were found to be affected in a manner similar to the condition of the others described above. In this instance, however, the apices were alone affected over an area in each of about the size of the palm of a hand. These were purplish-red, edematous, and contained small areas of solidification, which appeared grayish or gray in sections. There was a fibrinous exudate over the pleural surface of some. The other organs showed no change other than mild parenchymatous degeneration. The body was anemic. ¹

Cultures were made from the nodules in the lungs. On the original plate cultures two types of colonies were noted: One very small, similar to the typical colonies of *B. bovisepticus*, the other larger and with more tendency to spread, and upon slant cultures to coalesce. Both of these were studied.

The only cultural differences in these two strains were noted in agar, upon which one showed a greater inclination to spread and coalesce, and in broth in which one (464 ¹) caused a uniform clouding with a gelatinous sediment, while the other (464 ²) clouded the media less diffusely and formed flocculi, which were present not only in the body of the fluid but also on the sides and bottom of the tube. Both gave a brilliant indol reaction. Both were pathogenic for guinea pigs.

Sections for material fixed in Zenker's solution and imbedded in celloidin showed the same histologic picture as that described in above cases.

CASE V.

Calf No. 491 was received at the Serum Laboratory on February 24, 1904, and was given a prophylactic dose of 100 cubic centimeters of antirinderpest serum. A transient rise of temperature to 41.2° C. and a gradual return, during seven days, to normal followed this. On March 5, 1904, 5 cubic centimeters of virulent rinderpest blood was injected subcutaneously, upon which a scarcely perceptible rise of temperature lasting but twenty-four hours was noted. Four days later the animal was vaccinated and again the temperature rose, remained between 39° and 40° C. during the inoculation disease, and then gradually fell to normal. On March 25, 1904, a second injection, this time 50 cubic centimeters, of virulent blood was made, and this gave rise to a slight increase in temperature, which lasted but forty-eight hours.

During the following month the temperature remained above normal,

¹In the small intestine there were some round worms, which were preserved and sent to Chas. Wardell Stiles for identification. In his report he says that they are in all probability a new species of Hæmonchus.

varying from 39° to 40° C., and only dropped immediately before death. The animal died on May 5, 1904, a little over two months after its arrival at the laboratory.

At autopsy, done about eighteen hours after death, the upper halves and the anterior lobes of the lungs were found to be of mottled purplish-red color, and almost completely consolidated. In the areas of consolidation were a few nodules, generally of a pyramidal shape with their bases in the pleura.

The pleural surfaces of these were covered with a thin fibrino-purulent exudate, which upon removal showed the yellowish color of the nodule. On section the nodules appeared to be abscesses, surrounded by consolidated pulmonary tissue, and containing a thick yellowish pus.

Throughout the consolidated portions of the lungs there were smaller, almost miliary, firm areas. In general these were in the centers of lobules about which the trabaculæ surrounding them were increased in size and quite prominent.

The other organs showed no marked changes.

Cultures from the abscesses upon glycerin-agar and in broth showed no growth.

Histological examination of sections showed approximately the same arrangement in and about the nodules as in the previous cases.

CASE VI.

Calf No. 453 was received at the Serum Laboratory on January 12, 1904, and given 100 cubic centimeters of antirinderpest serum. The next day its temperature was 40.2° C., after which it fell to normal, remained so for three days, and then gradually rose to 40.8°, afterwards again gradually falling, the course covering a period of one week. From this time until death the temperature remained normal, except for a transient rise after vaccination and one following injection of 50 cubic centimeters of virulent rinderpest blood. It died on April 27, 1904, about three and one-half months after coming to the laboratory.

At autopsy the pulmonary lesions were similar to those found in No. 491, though less extensive, being almost limited to the apices and the extreme exterior borders.

Plate cultures from the nodules after twenty-four hours at 37° showed three slightly variant types of colonies. Transplants were made from each type and studied as B. 453,¹ 453,² and 453.³

After carefully comparing their appearances in and on various media, no other difference could be seen than that of size. B. 453 was a trifle larger, generally, than the other two. All produced typical colonies—i. e., the ones which were larger, more opaque, and which showed a slight tendency to coalescence.

These various differences between these strains will be discussed later. Suffice it to say now, that, in general the organisms in their cultural and morphologic characteristics were identical with *B. bovisepticus*.

Histologic examination showed a condition that agreed with that seen in previous cases.

CASE VII.

Steer No. 383 was received at the Serum Laboratory on January 6, 1904, and was given 100 cubic centimeters of antirinderpest serum. Two days later it showed some indisposition, and upon examination the temperature was found to be normal, the pulse somewhat accelerated, respirations rapid and shallow, coat stairing, appetite diminished, and rumination performed with indifference. Later the temperature became irregular with an evening rise which often reached 40° C. About ten days later, after the first examination, the animal developed a shallow, painful cough, which became more marked as the disease progressed. Tuberculosis, although up to that time unknown in these Islands, was suspected and the steer was killed sixty-one days after coming to the laboratory.

At autopsy a very widespread pathologic condition of the lungs was found affecting almost the entire extent of both organs. This consisted in a chronic bronchitis with bronchiectasis upon which a broncho-pneumonia had been engrafted. The bronchial walls were injected and covered with a sticky mucopurulent material. There were several bronchiectatic cavities, one the size of a goose egg, the others smaller, which had smooth fibroid walls within which were greenish-yellow muco-caseous masses of very foul-smelling material. The parenchyma of the lungs was studded with small areas of consolidation, some of which had undergone softening. The lungs were generally edematous. There were no marked changes elsewhere save congestion and cloudy swelling.

Cultures made from the small nodules at the time of autopsy showed no growths.

Tissues were preserved in Zenker's solution and embedded in colloidin. Sections from these materials were stained with hematoxylin and eosin, thionin, genetian violet (Gram and Weigert methods), and by the ones used to demonstrate tubercle bacilli.

The lungs on section showed an intense peri-bronchial infiltration, which was composed for the most part of cells of an epithelioid character with abundant protoplasm and vescicular nuclei. There was an increase in the connective tissue of these accumulations, which were also more or less regularly enclosed by fibrous capsules, making it appear as though the epithelioid accumulations had occurred between the mucous membranes and the surrounding muscular layers. Outside of the encapsulating layers were occasional lymphoid accumulations.

The mucous membrane of the bronchi was hypertrophied and corrugated—the cells being two or three layers thick and many of them vacuolated. The lumen was filled with mucous material in which numerous leucocytes were embedded.

In several places the mucous membrane on one side was hypertrophic, while on the other it was flattened and atrophied. Projecting into the lumen from the hypertrophied side were masses composed of small round cells and stroma, about whose internal edges were a row of large cells of peculiar appearance. One of these was an enormous cell whose protoplasm was very large in amount and whose nucleus was about 3 to 4

times the size of a poly-morphonuclear leucyte, and vescicular. The other ones were not as large, but were of the same type, except one large giant cell from whose position, and the arrangement, it is plain that it is formed by the coalescence of epithelioid (endothelial) cells. The remainder of the lumen was filled with cells similar to those described.

That the structures of the growth were granulomata might be seen in this same section, for the mixture of round epitheloid cells projecting into one large bronchus was well supplied with mature and immature blood vessels. There was no giant cell formation in these epithelioid or round-celled nodules. The only place, apparently, where this was found was on the lumen side of the projecting growths, and even here the occurrence was an exception.

The trabeculæ of the lungs were increased in volume. The perivascular connective tissue was increased and the parenchyma was injected.

It certainly seemed that in some places there was a tendency to syncytial formation in the mucous membranes of the bronchi, especially in those in which the peripheral growth had encroached largely upon the lumens.

In other sections the profuse fibroid overgrowth was most well marked. In such bronchi, so nearly obliterated that nothing but remnants of mucous membranes were to be seen embedded, masses of round and vescicular cells occurred. Giant cells could be seen in these, but here, too, these were nearer the peripheral margin of the nodules, and they seemed in a certain degree to have some relation to the smaller bronchioles upon which the cellular accumulations had encroached.

In other sections nodules could be seen whose centers evidenced commencing rhexis.

The liver showed a remarkable degree of fibroid change, evidently originating about the bile ducts. The tissue, besides its formative elements, containing many cells with vescicular nuclei. New gall-duct formations were also noticed.

No organisms were seen in the sections that resembled glanders bacilli, and there were no "acidfast" organisms discovered.

CASES VIII AND IX.

Calves Nos. 486 and 405.—Neither in their clinical symptoms nor in their general condition did these animals show any marked symptoms of disease. Their temperature kept within normal limits except after virulent blood injections and after vaccinations. They had no cough. They are up to the day of death.

In the case of No. 468, which became much weaker than did No. 405, appetite was present and the animal ate its fodder eight days after it had been unable to rise to its feet.

The lesions were comparable to those described in the other calves, but in both cases they were limited to the apices of the lungs. From the lesions of each animal an organism was obtained in cultures which was similar to the ones already described—viz, to B. bovisepticus.

The histologic examination showed approximately similar changes, although the processes were less advanced.

That these cases arose in the way Diekerhoff and Schneidemühl describe, there can be but little doubt. In certain animals, whose physical condition warranted slaughtering, we have noticed small areas of atelectiasis, which might or might not have been congenital, a point which we have been unable to determine. In certain others we have found both acute and subacute bronchial changes, with no macroscope parenchymatous changes. Whenever two such conditions coincided in the same animal the ideal opportunity would be afforded for the production of more serious pathologic complex, and we imagine that this is exactly what has happened in certain of these cattle.

It it an interesting feature of these cases that the bacilli of hemorrhagic septicæmia should be associated with so large a proportion.

Just what the duration of the disease has been in these animals can not be determined accurately. In the steer it was undoubtedly at least fifty days, and in the others it was probably thirty to ninety.

Infection has undoubtedly taken place in each case following the primary bronchitis, still later incipient pneumonic changes setting in.

Since the organisms present in the lesions proved to be the bacillus of hemorrhagic septicæmia, we may conclude that this is as common an inhabitant of the respiratory tract of animals here as it is in the United States.

Finally it may be said that from the above-mentioned facts that these cases are not examples of chronic infection with *B. bovisepticus*, but simply of an implanted infection in the course of other pathologic conditions, or perhaps in certain cases of a terminal infection. It seems probable, from our experience, that when primary infection with this organism occurs the disease runs a somewhat more acute course.

Another interesting fact was brought out in the bacteriologic inestigation of these cases—namely, that the cultural characteristics of the *B. bovisepticus* are not constant. Even with organisms of approximately equal virulence the growths on and in media vary considerably (these are perhaps most marked in bouillon as the accompanying table will show), and with races of unequal virulence the differences are still greater.

	1		
Agar.	Large colonies; tendency to confluence. Fine dewdrop colonies; no confluence. Confluent growth. Confluent growth of fine colonies; no confluence. Delicate growth of small colonies; no confluence. Do. Small colonies; no confluence.	Remarks.	Medium size; indol reaction brilliant. Small indol reaction; brilliant. Largest of nine races. Medium size; indol reaction brilliant. Very small. Do. Do.
Agar. Large colonies; tendency to confluence. Fine dewdrop colonies; no confluence. Moderately luxuriant, confluent growth. Very delicate growth of fine colonies; no Delicate growth of small colonies; no confluence small colonies; no confluence. Small colonies; no confluence.		Pathogenesis.	0 Guinea pig
	Large Fine o Moder Confly Very Delice Delice Small	Motil- ity.	0 0 00 00000
Gelatin lique- faction.	00000000	Gram's Motil-stain.	0 0 00 00000
Side of tube.	Precipitate do Precipitate Precipitate Precipitate	Peptone indol.	Positive ————————————————————————————————————
Fluid.	Diffusely cloudy. Cloudy flocculi. Diffusely clouded. Clear. Diffusely clouded. do. do. do. Clear.	Glucose bouillon.	No gas
Broth sediment.	Floceulo-viscid—do do do Viscid—do do d	Potato growth.	do
Pellicle.	Seanty Multish Seanty Whitish Go Go Go Go Seanty	Milk.	No changedododododododo.
No.	464 a 464 b 464 b 415 a 415 a 453 a 453 a 453 a 457 7	No.	464 b 464 b 453 a 453 b 453 b 453 c 453 c

It may be well to note here that, unless otherwise stated, the reaction of the media used is 1 per cent acid to phenolphthalein, and it was brought to this degree not by neutralizing with alkali and then adding hydrochloric acid, but by adding simply enough alkali to produce the right degree of acidity.

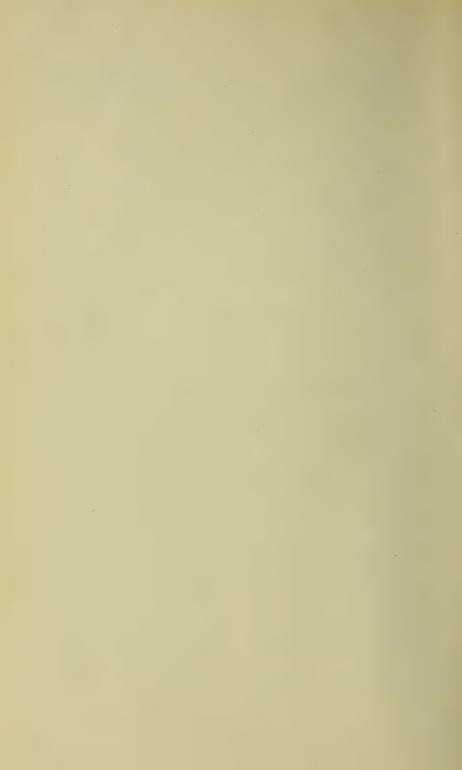
An excellent discussion of the cultural characteristics of the members of the bacilli of hemorrhagic septicamia will be found in Moore's book on 'The Pathology of Infectious Diseases of Animals."

A fuller discussion of the variations of *B. bovisepticus*, especially as regards pathogenicity, must be left for further study. From the few facts at the present time available we are not willing to draw conclusions.

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PART III.

REPORT ON PINTO (PAÑO BLANCO).

By Paul G. Woolley, M. D., Director of the Serum Laboratory.

Under the terms paño blanco, pinta, pinto, caraté, mal pintado, mal de los pintos, mal del pinto, peint, cute, cativi, quirica, pannus, carateus, and the spotted disease of Central America, is included a group dermatomycoses, characterized by peculiar nonpigmented patches on the skin, in the scales from which hyphæ or spores or both, of a mold-like fungus are found, which resemble in some cases Penicilium, in others Aspergillus, in still others Monilia.

Heretofore this epiphytic disorder has been reported from Mexico and Central and South America; another disease resembling it in some respects has been observed by Legrain in North Africa, and by Sandwith in Egypt, but, so far as I know, no previous report has come from the Philippine Islands.

The case which I wish to record is not the only one which I have seen in Manila, but it is the only one from which I have been able to obtain specimens for examination. All of the affected persons whom I have noticed have shown only the white variety, of which the following case is an example.

The history of the case is as follows: The patient was a Filipino laundryman, 15 years old, and in good health. There was no similar disease in any of his immediate family.

Upon inspection it was noticed that there were pinkish white patches, irregular in size and shape, on the ankles, dorsa of the feet, shins, knees, elbows, hands, wrists, and one on the right shoulder. This last-mentioned lesion the boy says was the one first noticed. The largest ones were over the external malleoli of the

ankles. These, the boy says, appeared after the one on the shoulder. The patches on the knees and elbows occurred later. None of these patches were of the same shape or size, nor were they definitely defined, but they shaded from their clear white centers to the normal brown of the skin. Neither were the lines of extension regular, so that the outlines of the patches were irregular and crenated. About the larger areas were smaller ones, some barely visible and of a faint pinkish white or very light-brown color.

On palpation it was evident that the skin over the larger patches was slightly rougher than the normal and that it felt somewhat thicker. The palpating finger could detect no abnormal variation in the covering of the smaller spots. There was but a minimum amount of scaling, and there was some itching.

The rate of extension had been extremely slow, for in three years the largest patch had a diameter of but 7 by 5 centimeters.

When asked regarding the cause, the boy said that the first spot came from carrying laundry baskets on his shoulder, and that the other ones followed traumata of one kind or another. There were no lesions on the palms of the hands or soles of the feet.

From one of the larger lesions on the ankle scrapings were made and examined in a solution of caustic potash (25 per cent). Among the epithelial cells branching, segmented hyphæ were seen forming a coarse mesh work. The mycelium was somewhat finer than that of Trichophyton; it was in general evenly refrangent, but in places beaded or granular. The spores were darker in color than the rest of the organism and less refractile. An occasional fructification was found in the smears, and in these the arrangement of the spores was like that of Penicilium. When treated with dilute fuchsin the spores were stained a very deep red. The hyphæ showed an inner segmented arrangement with a continuous inclosing capsule.

There was nothing in any of the preparations to suggest the description of Gastambide. The mycelial filaments were usually long, branched, and terminated in a bunch of spores. The description given by Montoya y Flores seems to apply more accurately to the fungus of this case.

There can be no doubt of the nature of the disease. The clear white spots with almost normal looking skin can be confused with no other skin affection with which I am acquainted. Diseases caused by trichophytons are extremely common in Manila and are

known generally as "dhobie itch," which is so common in the natives that in thirty cases of skin disease taken at random in Bilibid Prison twenty-four showed trichophyton filaments in caustic potash preparations.

It is possible that a brown pinta might be confused with pityriasis versicolor should the small patches occur on the face where it is said that the latter may occur. However, in the present case the clear white color of the irregular patches, the presence of sensation and of itching, together with microscopic findings, are enough to assure a correct diagnosis.

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PART IV.

NOTES ON ANALYSIS OF THE WATER FROM THE MANILA WATER SUPPLY.

By Charles L. Bliss, B. S., Chemist.

In December, 1903, it was suggested by the Superintendent of Government Laboratories and the Director of the Biological Laboratory that a systematic investigation, both from a chemical and bacteriological standpoint, be made of the water supplied the city of Manila. After careful consideration it was decided that this investigaton should consist of the usual sanitary chemical analysis and a count of bacteria, these to be made at regular intervals of one week and to extend over a period of several months. Later it was decided to examine the water for the presence of amebas and also to extend the investigation so as to include samples taken from the course of the watershed.

There were several reasons why such examinations were desirable:

- (1) Up to that time no chemical analysis of the city water had been made at the laboratory, and frequent requests for statements in regard to the water had to be denied.
- (2) It was supposed the water would vary considerably from time to time—not only from one season to another, but even from day to day. It seemed very probable that during the rainy season especially it would be subject to great changes within the course of a few hours, and that the results would be markedly different from the ones obtained during the dry, hot months preceding. This seemed all the more probable if any conclusions can be reached from the appearance of the water on different days. While ordi-

narily it is quite clear, a few hours after a rain it shows turbidity, which is very marked after a heavy storm. The watershed contains a quantity of suspended matter in the form of a fine silt, most of which gradually deposits when it is allowed to stand for a short time. This would naturally lead to the supposition that a variation could also be expected in the soluble constituents and in the number of bacteria.

- (3) Although numerous bacteriological examinations have been made of the city water since the organization of the Biological Laboratory, these had not previously been made at regular intervals, but only at random as opportunity offered; moreover, they were undertaken for different purposes. At times merely a count of bacteria was needed; at others, a search for certain specific organisms was undertaken, so that no continuous record was made of the number of organisms found in the water.
- (4) Amebas had been frequently found in the water in past times,¹ but it was not definitely known whether or not they were always present. With the prevalence of amebic dysentery in the city it was especially desirable to know whether these organisms were constantly present in the water.

The investigation was begun on December 14, 1903, and continued during seven months. It extended over the greater portion of the cooler weather, the hot season, and the beginning of the rainy season. At first weekly examinations were made; but after three months, there having been but little variation, examinations were made every alternate week. All samples were taken from a tap in the laboratory except on the following days: February 23, from El Deposito; February 29, from the Mariquina River at Santolan, and March 7, four samples from the Mariquina River (the watershed) at different points.

The taking of the samples (with the exception of those from El Deposito and Mariquina River), the methods of manipulation, the apparatus and chemicals were as nearly uniform as possible throughout the entire period, Monday of each week being selected as the day for doing this work. The last sample was analyzed on

¹Dr. Strong called attention to the presence of amebas in the water and to its unfitness for drinking purposes in his annual report as Director of the Biological Laboratory in 1902 and 1903. See Report of the Superintendent of Government Laboratories for 1902 and 1903.

July 15, for the reason that there had been an excessively heavy rainfall on the four days preceding, during twenty-seven hours of which time $17\frac{1}{5}$ inches of rain had fallen, the city and outlying districts having been completely under water from the evening of the 12th till a day or so later. A few weeks earlier an examination made eight or nine days after a rather severe typhoon showed results very nearly the same as those which had been obtained throughout the series; it was therefore thought best to take a sample very soon after the flood, rather than wait until the following week. It might be remarked that even in this instance there was but very little variation from the usual results.

Two samples were taken from one of the laboratory taps each Monday morning, after the water had been allowed to run for at least one-half hour; one was retained for chemical analysis, the other, with the usual precautions to prevent contamination, was sent to the Biological Laboratory. Both examinations were begun immediately. The sample for chemical analysis was collected in a 3-liter, glass-stoppered bottle, which was first rinsed thoroughly, filled and emptied two or three times, and then filled to the neck; the stopper was well rinsed and immediately inserted. This bottle was used for no other purpose throughout the series. The determinations of nitrites, nitrates, ammonia, and oxygen consumed were started at once so as to obtain results representing the true condition of the water before any changes due to oxidation, reduction, or bacterial action could vitiate it, all necessary precautions to prevent contamination by laboratory fumes being taken. As the results in chlorine and hardness would not be affected by any changes which might take place in the water, and as the residues would be but very little if at all altered, these determinations were deferred until opportunity to make them was at hand; but in every instance they were undertaken before the end of the week. In order to represent the water as it actually came from the pipes, all analyses were made with the unfiltered liquid.

The chemical analysis consisted of the determination of total residue; fixed residue; loss on ignition; nitrogen in the forms of

¹ The counting of the bacteria was done by Mr. Clegg, of the Biological Laboratory, and the determination of the presence of amebas was also done by Mr. Clegg in conjunction with Dr. Musgrave, who has compiled his results in Bulletin No. 18, Bureau of Government Laboratories, Biological Laboratory.

nitrites, nitrates, free and albuminoid ammonia; oxygen consumed; chlorine; also hardness. As some of the methods of manipulation were different from those usually employed, a brief description is given.

Total residue was determined in a platinum dish easily holding 100 cubic centimeters, by evaporation on the water bath, and heating for thirty minutes at 95° after drying. The dish, after the weighing of the total residue, was heated uniformly to low redness for three or four minutes. It was then placed in the desiccator and weighed as soon as cold. There was practically no change in the appearance in any sample on heating; at most only a very slight darkening and but little odor were perceptible, indicating the presence of only small amounts of organic matter. The loss on ignition was therefore due largely to decomposition of carbonate, as the heat was not sufficient to volatilize any chlorides.

Nitrites.—The reagents were prepared as follows:

- (a) Eight grams of naphthylamine hydrochloride were dissolved in water, 8 cubic centimeters of concentrated hydrochloric acid added, and the solution diluted to one liter.
- (b) Sulphanilic acid, a saturated solution in water containing 5 per cent concentrated hydrochloric acid. The test was made by placing 50 cubic centimeters of the water in a Nessler tube, adding 1 cubic centimeter of each of the above solutions, and mixing well by gently shaking. After thirty minutes the color was noted. In no instance did the depth of color indicate more than a very faint trace of nitrites, and very frequently none developed. Therefore a quantitative estimation could not be made.

Nitrates.—The aluminum reduction method was employed. After a few hours, when the reaction was completed, the ammonia formed, together with the free ammonia originally present in the water was estimated directly by the Nessler process; the free ammonia (determined in another portion of the sample) was deducted, the remainder being the ammonia formed from the nitrates. In the present series the determination of nitrates was begun within a few minutes after the sample was received, and the Nesslerizing was done on the following day. If nitrites are present in appreciable amount an allowance should be made for them.

A little more than 50 cubic centimeters of the sample were placed

in a 250 cubic centimeter glass-stoppered bottle; 2 cubic centimeters of sodium hydroxide of 33 per cent strength and 2 grams of aluminum filings were added, and the loosely stoppered bottle allowed to stand at room temperature until the next day. The solution was then filtered, with all precaution, into a tube, filling it to the 50 cubic centimeter mark. This solution was then Nesslerized, the necessary correction for free ammonia being made.

Free ammonia.—A round-bottom flask of 1 liter capacity with short neck was connected with a condenser 1 meter in length, the Nessler tube being slipped over the other end. On the day the sample was received the distilling flask was rinsed with distilled water; about 200 cubic centimeters of a solution of distilled water containing 1 gram of sodium carbonate were then added, and the greater part of the water distilled off, until the apparatus was free from ammonia. After cooling, 500 cubic centimeters of the sample were added to the residue, and the distillation continued at such a rate that a Nessler tube was filled to the 50 cubic centimeter mark in about ten minutes. Three tubes of 50 cubic centimeters each were collected and Nesslerized. As a matter of fact one tube would usually have been sufficient, at most two; for the third one never showed more than a slight trace of ammonia, and often none at all.

Albuminoid ammonia.—Fifty cubic centimeters of alkaline permanganate (8 grams of permanganate, 200 grams of potassium hydroxide, and 1,100 cubic centimeters of water, evaporated to 1 liter) were now added to the contents of the flask and distillation was continued, four tubes of 50 cubic centimeters each being collected. The distilling apparatus was used for no other purpose throughout the series, and it was always well protected from fumes.

Nesslerizing.—The Nessler solution was prepared according to the usual method and the standard solution of ammonium chloride contained (0.03812) gram of pure ammonium chloride in one liter. One cubic centimeter represents 0.00001 gram of nitrogen.

The distilled water in the laboratory was found to be free from even the slightest perceptible trace of ammonia; it was tested each time. A number of Nessler tubes were thoroughly rinsed with this ammonia-free water, then filled to the 50 cubic centimeter mark; portions of the standard ammonium chloride solution were measured in from a normal capillary pipette reading accurately

in hundredths of a cubic centimeter. The amounts used were 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.5, 2, and 2.5 cubic centimeters. Two cubic centimeters of the Nessler reagent were added to each, and also to the tubes containing the ammonia (free, albuminoid, and that from the nitrates). After allowing thirty minutes for development of the color, the comparisons were made. The results were expressed in terms of nitrogen per million.

Oxygen consumed.—The solution of pure potassium permanganate contained exactly 0.3953 grams in 1 liter. One cubic centimeter represented 0.0001 gram available oxygen, and the solution of pure ammonium oxalate was of exactly equivalent strength. These two solutions were kept in a dark closet and were standardized from time to time.

One hundred cubic centimeters of the water were measured into a 200 cubic centimeter Erlenmeyer flask from a pipette; 2 cubic centimeters of pure concentrated sulphuric acid were added, and then 10 cubic centimeters of the permanganate solution; the flask was then suspended in a boiling-water bath for thirty minutes. It was then taken out and 10 cubic centimeters of the oxalate solution were immediately added. The solution became colorless within a minute or so, and was then titrated with the permanganate till a faint pink color appeared, which remained permanent for a few moments. This determination was made in duplicate, two flasks being carried through at the same time. In no instance did the two titrations vary more than 0.05 cubic centimeters, and usually they were identical. For convenience, the titrations were made with a very narrow pipette graduated in 0.05 cubic centimeters. Results were expressed as parts of oxygen consumed per million.

Chlorine.—A solution of pure, recrystallized and dried silver nitrate was made containing 4.7940 grams in 1 liter. One cubic centimeter represented 0.001 gram of chlorine. A 5-per cent solution of potassium chromate freed from chlorides by precipitating with silver nitrate was used. The chlorine determinations were made in duplicate. Two portions of 250 cubic centimeters each were measured into casseroles of about 300 cubic centimeters capacity, carefully evaporated to about 50 cubic centimeters, 1 cubic centimeter of the chromate solution added, and the mixture titrated with the silver solution. By using one as a comparison

the first change in color from a pure to a reddish yellow was noted very sharply, and the two results never varied by more than 0.05 cubic centimeter; usually they were identical. A pipette similar to the one employed in the titration with permanganate was used. Results were expressed in terms of chlorine per million.

Hardness.—A solution of pure calcium chloride was diluted so that 1 cubic centimeter contained 0.0002218 grams, or represented the equivalent of 0.0002 gram of calcium carbonate. Fifty cubic centimeters of this solution should require exactly 14.25 cubic centimeters of standard diluted soap solution in order to form a lather which covered the surface of the liquid and persisted for five minutes. The soap solution was standardized each time and the proper value obtained, 50 cubic centimeters of the above calcium chloride being used for this purpose. This was compared with 50 cubic centimeters of the sample treated in the same manner, and the degree of hardness determined by reference to published tables. In the two or three instances, when the results varied from those usually obtained, and in all cases where any doubt as to the exact end-point existed the determinations were repeated.

Analyses of the Manila water supply.

[Results are given in parts per million.]

Laboratory No.	Location.	Date.	Total resi- due.	Fixed residue.	Loss on igni- tion.	Nitrites.	Ni- trates.	Free am- monia.
1745 - 1756 - 1780 - 1781 - 1803 - 1821 - 1840 - 1922 - 1944 - 1996 - 2025 - 2027 - 2028 - 2059 - 2157 - 2225 - 2303 - 2352 - 2384 - 2444 - 2447 - 2487 - 1780 - 17	Deposito Santolan Mariquina River do do do	Feb. 29, 1904 Mar. 7, 1904	220 181 188 188 191 179 176 162 168 164 163 174 173 174 173 174 173 174 174 175 180 165 196 174 178	190 148 152 142 160 148 1552 150 152 152 152 145 138 134 127 147 145 138 136 136 135 150 138	30 33 36 46 31 31 24 26 20 16 23 26 26 27 28 29 26 35 34 46 36 29 35 34 35 36 36 36 36 36 36 36 36 36 36 36 36 36	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0.150 .271 .100 .112 .080 .119 Trace. .100 .292 .124 .139 .112 .136 .220 .220 .158 .200 .158 .200 .360	0.0079 .0049 .000 .008 .002 .002 .002 .006 .028 .036 .028 .036 .021 .024 .024 .024 .024 .024 .024 .024 .025 .026 .027 .026 .027 .027 .028 .038 .038 .038 .038 .038 .038 .038 .03

Analyses of the Manila water supply—Continued.

[Results are given in parts per million.]

Labo- ratory No.	Locati m.	Albu- min am- monia.	Oxy- gen.	Chlo- rine.	Hard- ness.	Bacte- ria.	Ame- bas.	Remarks.
1739		0.078	1.90	2.13	85.7	400	Billio sea	Heavy rain dur- ing few days preceding; water very turbid.
1745 1756		. 073	1.85 .875	2. 23 2. 60	85.7 90.0	550 600		Do. Water distinctly
1780		. 052	. 86	2.40	94.3	460		turbid. Water slightly turbid.
1781		. 034	. 85	3.00	101.5	250		Do.
1803		. 038	. 65	3.04	94.3	200	_	Do.
1821		. 044	. 65	3.00	87.1	200	-	Do.
1840		.048	. 90	3.00	95.0	150		Water almost perfectly clear.
1880		. 048	. 90	2, 60	104.0	125	+	Do.
1922		. 044	. 95	3.20	95.0	150	+	Do.
1944	Deposito	. 052	. 95	3, 30	109.0	120	+ + (4) 0	
1996 2025	Santolan Mariquina	. 086	1. 25 1. 60	3.40	97. 0 97. 0	112	+	All contained
	River.					v	,	deposit, apparently organic matter and silt. Determinations of residue were made with filtered water. Each gave slight dark color and odor on ignition.
2026 2027 2028 2069 2157 2225 2303 2352 2385	do	. 080 . 062 . 060 . 048 . 052 . 062 . 048 . 064 . 042	1. 50 1. 07 1. 35 1. 00 . 82 1. 07 1. 20 1. 20	3. 20 3. 60 3. 60 3. 88 4. 14 3. 80 4. 40 4. 20 4. 40	93.2 93.2 94.8 95.0 101.8 97.8 95.0 98.0 92.0	208 105 267 120 120 108 125 175 130	+ + + + + + + + + + + + + + + + + + + +	Do. Do. Do.
2434		.074	1.60	4. 20	91.0	206	+	Heavy rains just previous; wa- ter turbid; slight sedi- ment. Slight- ly dark color and odor on ignition.
2464		. 040		36.0	83.0	135	+	No rain immediately preceding.
2487		. 050	1.70	2.80	71.4	450	+	Very turbid. Heavy rains.
2511		. 068	2, 20	3.16	58.8	(5)	+	Very turbid.

The counts of bacteria and determinations of the presence of amebas were made by Dr. W. E. Musgrave and Mr. M. T. Clegg, both of the Biological Laboratory.

¹ Very faint trace.
² Faint trace.
³ Distinct trace.
⁴ Not sufficient sample. A sample taken following week, bacteria, 100; amebas, 0.
⁶ Rapid growth over surface prevented count.

On examining the above table the following maxima and minima appear during the period covered by the report:

	Minimum.	Maximum.
Total residue	. 031	220 190 46 Trace. . 36 . 036 . 100 2. 20 4. 40

As will be seen despite the great variation in the weather conditions the differences in analytical results were not very great. times the chlorine ran as high as 4.40 indicating some contamination, but these maxima were only transitory. However, a water may show a very high degree of purity, so far as this can be determined by chemical analysis, and yet be unfit for drinking purposes because of either the large number of micro-organisms which it contains or because of their nature; so that it may be possible to convey the etiological factor of typhoid fever, cholera, dysentery, etc., by a drinking water which chemically would be pronounced unobjectionable. The chemical analysis may indicate a probable pollution with sewage or with other matter which may be suspicious or dangerous at all times, and it may condemn such a water. However, in the case of the Manila supply the long series of analyses gave such results that no one would be justified, even at the worst, in stating from a chemical standpoint that this water was either injurious or deleterious to the public health. For this reason, as has been repeatedly pointed out by others, a bacteriological examination is essential.

However, in glancing over the number of bacteria it will be seen that the maximum, on December 28, was 600, and this large number was quite unusual, the average being below 250 organisms to 1 cubic centimeter and in many cases even below 150. Bacteriologically, therefore, the water may not be regarded as very suspicious, especially since the general series of determinations did not demonstrate pathogenic organisms to be present, and indeed typhoid fever is of but rare occurrence in Manila. During the cholera epidemic it does not seem likely that any of the cases could have been referred to direct infection from the Manila water supply.

A factor however, which, apart from the chemical and bacteriological analysis, is the most important, is shown in the last column,
where it is demonstrated that amebas, whether pathogenic or not,
are almost constantly present in the water supply, and Dr. Musgrave and Mr. Clegg, of the Biological Laboratory, in Bulletin
No. 18, "Amebas: Their Cultivation and Etiologic Significance,"
have shown that amebic dysentery can be sometimes produced in
monkeys by cultures made from the water supply. Neither a
chemical analysis nor a bacteria count will demonstrate the presence
of these dangerous factors of disease, and consequently, in the
Tropics at least, if we wish to obtain a fair idea of the condition of
the water, a determination of the presence or absence of amebas is
necessary.

The ordinary sanitary analysis does not include an examination for substances which in themselves are injurious. The quantities of ammonia, nitrate, chlorine, etc., found in an ordinary water are harmless—they are simply indicative of a possible pollution, but before judgment can be passed in regard to the sanitary analysis, the source of the water, the geological conformation of its surroundings, and so forth, must be taken into consideration. An amount of chlorine, for example, which would be perfectly normal in water from one locality might indicate contamination with sewage in that from another. The total residue in this series was always below 200 parts per million excepting on one occasion which was after a heavy rain.

Very little darkening of the residue on heating was noted in any sample; sometimes there was none, so that in this respect no criticism can be made of the water. The presence of nitrites in measurable quantity is sufficient ground for condemning a water as a rule, for nitrites indicate bacterial action. However, in this water nitrites were frequently absent altogether, and only on one occasion were there more than a trace, this occurring immediately after a heavy rain.

The amount of nitrates was always very low. The highest amount of free ammonia found was 0.008 per million, excepting in five samples taken from the Mariquina River itself where the results varied from 0.0021 to (0.036), so that by the time the water from the river reaches Manila the ammonia has largely become oxidized to nitrate. This is borne out by the fact that the amount of nitrate in those samples which were taken on March 7 was

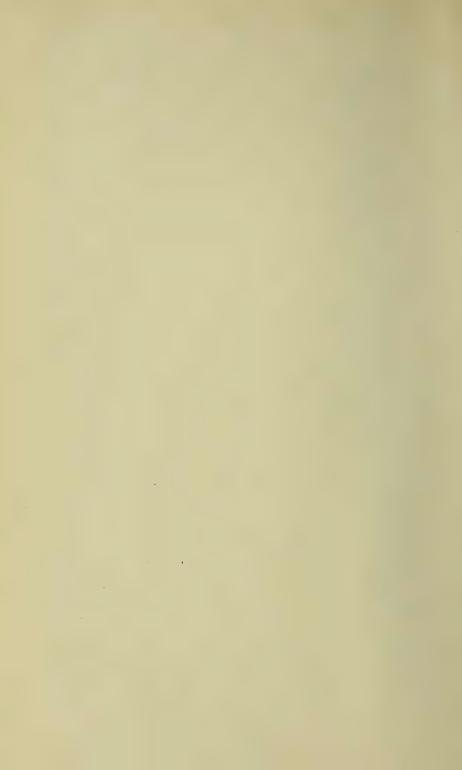
lower than the usual quantity obtained. Albuminoid ammonia, as a rule, was also quite low. None of these factors, therefore, would indicate a contamination of the river water.

The same may be said of the amount of oxygen consumed. No figure exceeding 2 was found excepting in one instance, and that was three days after the great flood in July. The amount of oxygen consumed seemed to be greater immediately after heavy rains.

The low results obtained in the investigation of chlorine are favorable indications as to the quality of the water. One would expect rather higher chlorine values in the waters of the Philippine Islands owing to the proximity to the sea, but this is not as a rule the case, as analyses of waters from other localities have demonstrated.

After heavy rains more or less insoluble suspended matter, both inorganic and organic, is rinsed into the supply. However the turbidity of the Manila water is generally due to a very fine silt which has its rise in some of the clay beds at the source of the river, and is for this reason harmless. Therefore the analyses made in the Chemical Laboratory show that the water supply of the city of Manila is of a very good quality, but the constant presence of amebas, as demonstrated in the Biological Laboratory, render the water unsafe for drinking purposes unless it is boiled.

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PART V.

FRAMBOESIA: ITS OCCURRENCE IN NATIVES OF THE PHILIPPINE ISLANDS.

By Paul G. Woolley, M. D., Director of the Serum Laboratory.

In a recent visit to Benguet Province, in the central part of northern Luzon, I became interested in a peculiar disease which was called "Lepra" by the native Igorrotes, this term having been taught them by the Spaniards. Inasmuch as I was entirely unprepared for a complete study, and because it is extremely difficult to obtain any history or to make complete examinations of these people, the following report will be meager, but it is interesting, since I feel certain that the disease is one closely related to if not identical with frambœsia.

In Baguio, Benguet, I saw, with Dr. Thomas of the Civil Sanitarium, two cases; one, a woman aged about 35 years, and her son, the latter of some 15 years, both of whom presented small raspberry growths upon the face. In the mother, these were situated at the corners of the mouth; in the son, in the nasolabial folds, and they much resembled the growth pictured in the New Sydenham Society's Atlas (Fasc. XIV, Pl. B. fig. 8). Both of these persons showed pigmented scars on the neck and face. At a later time I searched for these two people in their native town of Agno, Benguet, but was unable to find them.

The case from which I procured the tissue which I will describe below, I saw one morning as Mr. Barron—the sanitary inspector of the province—and I were returning from a long trip in the mountains. As we stopped to rest at a little native village, we noticed that several of the children had peculiar looking, sluggish ulcers on their legs, necks, or bodies, and also showed signs of considerable anemia. We asked concerning this affection and were told that the Spanish called it "Lepra," and that many people had it. This was all we could learn, except that the sores were not painful, though they showed the effects of scratching, and that they eventually healed. One grown person, a woman, was found who had similar lesions on her neck. Whether she had others elsewhere we were unable to discover. One lesion on the neck (see Sydenham Society's Atlas, Fasc. XIV, Pl. LXXXV, upper left-hand corner of lower figure) was a shallow ulcer with a firm grey base, a well-defined, firm margin, a slight yellowish secretion, not surrounded by any appreciable induration, not painful, and situated on the neck just below the angle of the jaw. This was excised and preserved in commercial alcohol. Near it were some pigmented areas, somewhat darker than the normal skin, not appreciably thickened, and possessed of sensation, and which the woman said were at the sites of healed ulcers similar to the ones we saw. (New Syden. Soc. Atlas, Pl. LXXXXV.) An infant which the woman carried had similar lesions on its legs, face, and neck. Another child of the same woman, which, however, we did not see, was said to be afflicted in the same manner.

The gross appearance of the lesions would lead one to think of leprosy, tuberculosis, epitheliomia, syphilis, or yaws. Leprosy I think may be excluded. There were no cases of outspoken leprosy among the persons of the pueblo in which these persons lived. There were no anesthesias or leucoplakias in any of the cases examined. The histologic examination was negative. Tuberculosis could be excluded since neither the lesions nor the scars had the classic appearance of lupus, nor was the histologic evidence sufficient to support such a diagnosis. Epithelioma could only be excluded by microscopic examination. All in all the cases seemed most like syphilis. None of the people of the pueblo showed outspoken signs of this disease, although, as stated, a complete examination could not be obtained. The inhabitants of this region generally are rigidly moral and rigorous punishment is inflicted upon any who overstep the bounds. But while they live morally clean lives, their physical surroundings are filthy, which may account for the modification of the frambœsial lesion and the predominance of

infection. Treatment, of course, has not been tried, so one can not say what effect mercury or iodides might have, but from the evidence that I have at present I incline to the idea that the cases seen were examples, not of syphilis, but of frambœsia.

The tissue was embedded in celloidin, and the sections which were made later were stained with hæmatoxylin and eosin, Unna's polychrome blue, Gram's stain, and by the tubercle bacillus methods. Microscopic examination showed that the lesion consisted of a marked hyperplastic acanthosis, with a round-celled infiltration of the underlying, and especially of the perivascular, connective tissues. The first impression was that one was dealing with an epithelioma, but closer examination showed this to be a delusion. The acanthus layer of the skin was thickened and prolonged in strands and columns of bizarre shapes. In many places in this hyperplastic epithelium there were larger or smaller islands of connective tissue, each, apparently, representing the path of a blood vessel. In the centers of such areas and about the vessel were collections of small round and plasma cells, but this smallcelled infiltration was most marked in the larger strands of the submalpighian connective tissue. Here the increase of these formative elements was remarkably prominent, and there was throughout the sections the same perivascular arrangement. Within these areas there were occasional leucocytes; there were also fibroblasts in varying stages of development, and a number of plasma cells were present within the round cell accumulations. At the site of the ulceration the structure of the lesion was modified by the destructive process. Here all the layers were invaded by a multitude of polymorphonuclear leucocytes, the blood vessels were widely dilated, and there was a certain amount of superficial degeneration, but the structure of the lesion in the not degenerated parts was still perceptible. So far as the arrangement of the layers of the skin was concerned there was no distortion. In many parts of the sections a peculiar appearance was seen which gave one the same impression as that produced by the scales of a fish, or by the overlapping of shingles upon the roof of a house. This was apparently due to the fact that certain of the acanthus cells took a more intense stain on one side. In this phenomenon (seen best in polychrome blue specimens), the nucleus did not participate. In none of the sections, and these included the whole of the lesion studied, were there any giant cells, tubercle of lepra bacilli, and no evidence of cell inclusions was seen.

As for the occurrence of such lesions in frambæsia, and their distribution, little can be said excepting as quotations from authors who have had considerable experience with the disease. Manson says, in discussing the distribution of the vaws, that they may be scattered over the entire body, or the crop may be limited to one or two growths, or they may be confined to a circumscribed region of the skin. Moreover, there may be successive crops evolved, especially when the person affected is debilitated. Morris remarks that the disease in adults is more chronic than in children. When the yaw develops normally it does not ulcerate, but Manson says that the tumors instead of being absorbed, may break down and ulcerate, the ulceration usually being confined to the yaw itself, although it may go deeper and give rise to extensive sores. With the development of the deeper and more extensive forms of ulceration, the typical lesion of frambæsia may disappear for a time, or perhaps permanently. If such is the case, the ulcerations are said not to be infective and to not communicate the disease, although they may persist for years. Nicholls, quoted by Mason, states that ulceration occurs in about 8 per cent of the cases. There is no histologic description of the variety of the lesion which I have encountered to which I can refer, though from Unna's and Charlouis's description of the typical yaw it is but a modification of the latter. These writers speak of the true yaw as a cutaneous plasmoma complicated by epithelial hyperplasia and hyperkeratosis. Except that the lesion described above is not raised it certainly corresponds in many details with Unna's and Charlouis's description.

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SECTION STAINED WITH HÆMATOXYLIN AND EOSIN. (ZEISS COMP. OC. 6, OBJ. A. A.)

This shows the hyperplasia of the acanthus layer of the skin, the dilated blood vessels, and the perivascular accumulations of small round and plasma cells. Photomicrograph by Martin.



No. 21.-OCTOBER, 1904

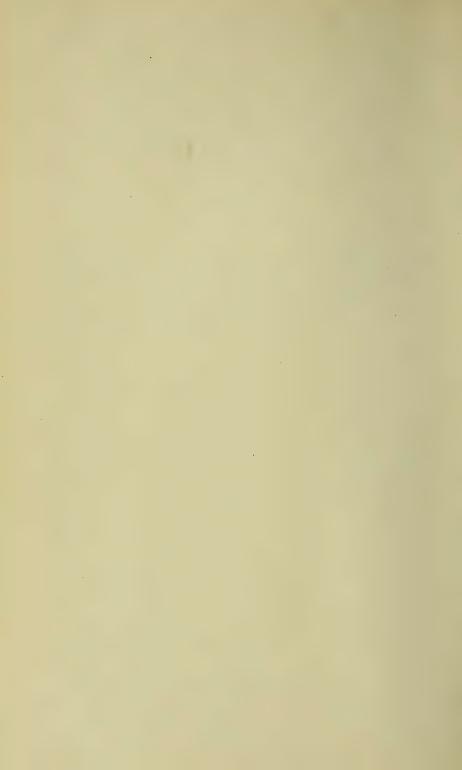
DEPARTMENT OF THE INTERIOR BUREAU OF GOVERNMENT LABORATORIES BIOLOGICAL LABORATORY

SOME QUESTIONS RELATING TO VIRULENCE OF MICRO-ORGANISMS, WITH PARTICULAR REFERENCE TO THEIR IMMUNIZING POWERS

BY

RICHARD P. STRONG, M. D.

MANILA
BUREAU OF PUBLIC PRINTING
1904



LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila July 15, 1000

Manila, July 15, 1904.

SIR: I have the honor to transmit herewith and recommend for publication a paper, entitled "Some Questions Relating to the Virulence of Micro-Organisms, with Particular Reference to Their Immunizing Powers," by Richard P. Strong, M. D., Director of the Biological Laboratory.

I am, very respectfully,

Paul C. Freer,
Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER, Secretary of the Interior, Manila.



SOME QUESTIONS RELATING TO THE VIRULENCE OF MICRO-ORGANISMS, WITH PARTICULAR REFERENCE TO THEIR IMMUNIZING POWERS.

By RICHARD P. STRONG, M. D., Director Biological Laboratory.

The experimental work which forms the basis of this article was for the greater part performed during the spring of 1903, in the Institut für Infektionskrankheiten, Berlin, Prof. R. Koch, director, department of Prof. A. Wassermann. I wish here publicly to express my very grateful thanks to Professor Wassermann, under whose direction these studies were first undertaken, for many suggestions and courtesies. I also wish to express my gratitude to my colleague Dr. Paul C. Freer for having read the manuscript.

During an experimental study of protective inoculation against Asiatic cholera it became desirable to conduct experiments with two strains of cholera spirilla of different degrees of virulence. Throughout the course of these studies, which extended over a period of a number of months, there often arose in my mind the question of the essential differences existing between these two stems, particularly in relation to the subject of their virulence and to the immunity to which they gave rise in inoculated animals. The investigation of these questions forms the basis of this paper.

For the description of the two strains—their source, identification, etc.—the details of the technique employed in the experiments, and other particulars, the reader is referred to a previous article (1).

It will be sufficient to state here that some time was spent in accurately standardizing these cultures, and the minimal lethal dose for guinea pigs of 250 grams' weight was carefully determined. After numerous passages of "virulent" through animals,

¹For the sake of brevity these strains will, in this article, be designated as "virulent" and "avirulent."

the lethal dose of 0.1 of a standard (2 milligrams) oese 1 of a twenty-hour agar culture was reached. Such a dose of "virulent," when suspended in 1 cubic centimeter of an 0.85 per cent sodium chloride solution and injected intraperitoneally into a guinea pig of 250 grams' weight, regularly caused death within twenty-four hours; with "avirulent," on the other hand, 1½ standard oesen of a twenty-hour agar culture, when injected intraperitoneally, were required to produce death within the same time in such an animal. The former stem, therefore, may be said to possess fifteen times the virulence of the latter.

Throughout the course of the work this relation of virulence was carefully maintained. As the virulence of cholera spirilla which have been grown upon artificial media quickly changes, it was necessary in the case of the virulent organism to make daily animal inoculations and always to use the same generation of the strain. With the avirulent culture considerable care was also required to keep it at the minimal lethal dose of $1\frac{1}{2}$ oesen.

TECHNIQUE.

The technique of the agglutinative and bactericidal reactions employed throughout the work was as follows:

The reactions for agglutination were performed in the test tube. One oese of the living organism was thoroughly suspended in 1 cubic centimeter of an 0.85° per cent solution of sodium chloride. The amount of serum to be tested suspended in 1 cubic centimeter of a similar saline solution was then added, the tube thoroughly shaken, and the mixture placed for two hours at 37° C. In a complete agglutination it is understood that the liquid overlying the precipitated bacteria appears entirely clear. By a weak reaction we understand one in which there is a distinct agglutination with precipitation, visible to the naked eye, of numbers of the organisms, but in which the supernatant fluid remains more or less cloudy.

The bactericidal reactions were performed in the abdominal cavity of guinea pigs according to the well-known method of R. Pfeiffer, a hypodermic syringe with a blunt-pointed needle being employed for the injections, and care being taken to avoid any injury to the intestine during the inoculation. The dilutions of the serum were made in normal saline solution. One cubic centimeter of the diluted serum was then added to 1 cubic centimeter of bouillon containing 2 oesen of "virulent" in suspension, after which 1 cubic centimeter of the resulting mixture was injected into the peritoneal cavity of a guinea pig of 250 grams' weight (or a little less), the animal thus receiving ten times the fatal dose of

¹ This standard oese was employed throughout the work.

living organisms. A fresh guinea pig was used for each reaction. The experiment was controlled by microscopic examination of a drop of serum from the abdominal cavity, made immediately and again twenty minutes after the inoculation, and obtained by means of a capillary tube, and by the inoculation of control animals with ten times the fatal dose of "virulent" without serum. The result to the animal after twenty-four hours, whether living or dead, was regarded as the final test, though the condition of the organisms in the abdominal cavity after twenty minutes was always carefully noted.

With these explanations we may now turn our attention to the study, between the two stems, of certain of the essential differences in relation to virulence.

By the virulence of a micro-organism we have come to understand its pathogenic capabilities—that is, the extent to which it may harm a susceptible host. The virulence of a bacterium, therefore, represents the sum of its specific injurious influences upon such a being. Some authors differentiate between virulence and toxicity, (2) including in virulence only the infectious capability of the organism—that is, the power to grow and to multiply in the animal body—and in toxicity the ability to produce a specific poison, and the amount of such poison. However sharply such a distinction may be observed in diphtheria and tetanus, in which diseases the infectious process (that is, the propagation of the living bacteria) and the intoxication process (that one due to the action of the soluble nonviable substances set free from the organisms) are quite distinct, and while it is well known that certain strains of diphtheria bacilli may at the same time possess strong infective and but little toxic power (and vice versa), in Asiatic cholera the two processes of infection and intoxication are apparently so closely intermingled that, although theoretically a sharp distinction is possible, practically, in the study of the infection of the animal organism, it is only with difficulty that the two are actually differentiated. Hence, in considering the virulence of a strain of living cholera spirilla, both its infectious and its toxic power may, to a certain extent, be regarded conjointly.

Until recently our ideas as to what specific properties the virulence of an organism depends upon have been very vague and indefinite.

¹With the exception, perhaps, in diphtheria of the intracellular toxine of Welch and Flexner concerned in the production of the false membrane.

Kruse, (3) in his earlier ideas regarding the theory of infection and immunity, believed that a certain analogy existed between the virulence of micro-organisms and their ability to produce enzymes. He conceived the hypothesis that pathogenic bacteria possess a certain dissolving power ("lytische Kräfte"), through which they are able to bind and to paralyze the opposing bodies (alexines) of the living organism. A loss of virulence was therefore supposed to go hand in hand with a loss of these dissolving substances (or Angriffsstoffe).

Smirnow (4) pointed out that cultures which show a loss of potency in their vital manifestations (especially in their virulence) also present simultaneously a diminished energy of growth. Thus, he maintained that the diameters of colonies of virulent anthrax bacilli are from two to four times as great as those of avirulent strains of the same organism. However, he shows that the diminished virulence of a bacterium depends not alone upon the loss of some one specific property but upon a real degeneration of the organism, which manifests itself by a diminished energy of growth and a greater susceptibility to damaging influences.

Behring, (5) in working with different strains of anthrax bacilli, in general confirmed Smirnow's observations. He further pointed out that in cultures of this organism the production of acid and the power to reduce litmus increase with the virulence of the strain.

Gotschlich and Weigang (6) maintain that the virulence of a cholera culture depends exclusively upon the number of living individual spirilla which it contains. According to their idea, the virulence of an organism would depend upon its power to multiply within a given time, that is, the more virulent the organism the shorter the interval between successive generations.

Beyer (7) found that if a small piece of silver foil was placed upon the surface of an inoculated agar plate the more virulent the organism the narrower was the clear zone between the growth and the silver foil. In case the organism was very virulent, the growth was said to extend so as to touch the margin of the silver.

Marx and Woithe (8) maintained that the virulence of a microorganism in a human or an animal infection can be judged by the number of bacteria which contain *Babes-Ernst's* bodies. The more numerous such organisms are in a disease or in a culture, the greater is its virulence. However, Ascoli (9), Krompecher (10), and Gauss (11) were not able to confirm these observations. Thus, Gauss, with a culture of *Bacillus pyocyaneus*, which had been passed many times through animals, obtained a virulence forty times as great as that of the original strain. Yet he was unable to find *Babes-Ernst's* bodies in a single organism of this culture, which possessed the highest obtainable virulence.

Pfeiffer, (12) in 1897 found that in immunization against plague the degree of immunity produced depended not only upon the dose of the killed pest culture but also upon the degree of virulence of the killed organism. He showed that an ape, which had been given a single injection of a virulent agar culture, carefully sterilized by heating, was later protected against 1 oese of the virulent bacterium. If, however, an avirulent strain of the pest culture was employed for the virus, the animal was not protected against the same amount of the virulent germ. Therefore Pfeiffer concluded that the immunizing effect of pest bacilli is up to a certain degree proportional to the virulence of the culture employed. Kolle and Pfeiffer, (13) in 1895 also demonstrated that a virulent typhoid bacillus required many more times the amount of immune serum to bring abount its bacteriolisis in the abdominal cavity of a guinea pig than did the less virulent strain. In 1896 these authors (14) pointed out that an immune serum agglutinated a less virulent cholera organism in much higher dilutions than a virulent one. Pfeiffer (15) continued his researches in this direction, and further investigated with Friedberger (12) the question of the virulence of the cholera vibrio. They concluded that in the case of the killed cultures of this organism the immunizing effects were also proportional to the virulence of the inoculated strain. From these facts they drew the conclusion that the virulent and the avirulent organisms differ in the number or degree of affinity of their haptophore groups; and demonstrated this conclusion by experiments in which it was shown that in a goat's cholera immune serum a virulent organism bound many more times the number of amboceptors than certain avirulent ones.

These last experiments of R. Pfeiffer and Friedberger seemed of such great importance in connection with Ehrlich's hypothesis that it was decided to repeat them, and, in addition, to perform them in as accurate a comparative way (with relation to the virulence of the strain) as practicable. This seemed desirable because in Pfeiffer's and Friedberger's work, as far as can be ascertained from their article, no attempt was made previously to determine the exact relationship of virulence of the different stems to one another, it being apparently the authors' idea in these experiments merely to show that the virulent organism always bound more amboceptors than the less virulent ones; at any rate, the exact relationship between the virulence and the power of binding amboceptors was not emphasized.

Moreover, Pfeiffer's and Friedberger's absorption experiments above referred to were performed with the serum of immune goats, while the bactericidal reactions were performed in the abdominal cavity of guinea pigs. Since at this time it was not established whether the receptor structure of the cholera vibrio for both of these animals was identical, and whether the difference in virulence between the two strains was relatively the same for each animal, and since it was even disputed whether their intermediary bodies were identical, it was thought desirable to perform these experiments also with guinea-pig serum. This work was undertaken in the following manner:

ABSORPTION EXPERIMENTS WITH THE LIVING ORGANISMS AND GUINEA-PIG IMMUNE SERUM.

A large guinea pig was inoculated subcutaneously with 2 cubic centimeters of an aqueous solution containing the free receptors of the virulent cholera spirillum obtained by the autolytic digestion of the organism,² and eight days later was reinoculated intraperitoneally with 2 oesen of the living virulent strain. After another eight days it was killed by bleeding and the bactericidal value if its serum carefully determined. This was found to be about 0.11 milligram. Separate portions of the serum were then

¹ Since these experiments were performed Pfeiffer and Friedberger (16) have shown that goats' and rabbits' cholera amboceptors unite with the same receptors of the cholera spirillum.

²This solution constitutes our cholera prophylactic. For a detailed description of its preparation, etc., the article on protective inoculation against Asiatic cholera (1) above referred to should be consulted.

diluted with normal saline solution in the proportions of 1:20 and 1:100. Four centrifuge tubes (designated for convenience as A, B, C, and D) were then taken. Two of them, A and B, were filled with 5 cubic centimeters of the serum diluted in the proportion of 1:20; the other two, C and D, with 5 cubic centimeters of the serum diluted in the proportion of 1:100. Five oesen of the living virulent organism were then suspended in the serum of each of tubes A and C, and 5 oesen of the living avirulent organism in each of the two remaining ones, B and D. After a thorough mixing of the contents, the tubes were placed for two hours in the ice box. This time was allowed for the binding reaction between receptors and amboceptors to become complete, and the object of keeping the tubes at a low temperature was to prevent any further multiplication of the spirilla. Upon removal of the tubes from the ice box the agglutination of the organisms was apparently complete in all of them. After thorough centrifuging, the clear fluid above was pipetted off and in each case carefully examined for its bactericidal value in the usual manner. Table IX shows these results calculated for 1 cubic centimeter of the undiluted serum.

From a study of the serum diluted to 1:20, we see that the portion treated with the virulent strain afterwards showed a bactericidal value of 1:500 or about one-seventeenth of that treated with the avirulent one (1:8,500), and upon subtraction of these values from that of the original serum (1:9,000), we see that the virulent organism has bound about $\frac{8.5}{9.0}$ and the avirulent one about $\frac{5}{9.0}$ of the amboceptors present. In the case of the serum diluted to 1:100, the bactericidal value of the portion treated with the virulent strain (1:600) was about $\frac{1}{14}$ as great as that treated with the avirulent one (1:8,500), the ratio of absorption being as $\frac{8}{9.0}$ to $\frac{5}{9.0}$. Hence, evidently in the cholera-immune serum the virulent organism had usually bound from sixteen to seventeen times as many bacteriolytic amboceptors as the avirulent one; or the haptophore groups of the former spirillum had shown from sixteen to seventeen times as great a power of absorption as those of the latter.

The results of these experiments, showing the values of the sera expressed in units of immunity for 1 cubic centimeter of the

¹ The value of the serum in Tube B (see Table IX) varied with different animals between 1-8,000 and 1-8,500. If we regard 1-8,000 as its value then the ratio of absortion in Tubes A and B is as \$\$ to \$\$ or 8.5:1

diluted serum, and the number of units absorbed per cubic centimeter may be seen in the accompanying table:

Dilution of theserum to which the bacte- ria were added.	Original value of 1 cubic cen- timeter of the dilut- ed serum.	Value of 1 cubic centimeter of the diluted serum after the absorption of 1 oese per cubic centimeter of—		Number of units absorbed per cubic centimeter by 1 oese of—	
		Virulent.	Aviru en .	Virulent.	Avirulent.
1-20 1-100	450 90	25 6	425 85	425 84	25 5

¹In units of immunity. By 1 unit of immunity we understand the amount of serum which will protect a guinea pig of 250 grams' weight against ten times the fatal intraperitoneal dose of living cholera spirilla. (Throughout the course of the work only the "virulent" organism was employed in testing the bactericidal power of the sera.)

ABSORPTION EXPERIMENTS WITH THE LIVING ORGAN-ISMS AND RABBIT'S IMMUNE SERUM.

Experiments were then performed in a parallel manner with rabbit immune serum, which was obtained in the following manner: A rabbit was inoculated with 6 cubic centimeters (1 cubic centimeter contains the number of receptors obtained from 8 oesen) of the heated virulent cholera prophylactic. Eight days afterwards it was killed by bleeding, and its blood serum upon examination was found to have a bactericidal value of 0.04 milligram. Five oesen of the living virulent strain were now carefully suspended in 5 cubic centimeters of this serum in the following dilutions: 1:20, 1:100, and 1:500. Five oesen of the avirulent organism were also added to each 5 cubic centimeters of the serum in the same dilutions. The mixtures were placed in the ice box for two hours. Upon removal it was found that in the tube which contained the serum in the dilution of 1:500 and the virulent organism agglutination was not entirely complete, and that although the overlying liquid was nearly clear it was not wholly so, and evidently still contained some organisms. In the other tubes complete agglutination of the bacteria had apparently occurred. The mixtures were then carefully centrifuged and the overlying liquid pipetted off and in each instance examined for its bactericidal value by the usual method. The results are recorded in Table X (as calculated for 1 cubic centimeter of the undiluted serum), and are in general similar to those obtained in the case of the guinea-pig serum. The values of the sera expressed in units of immunity for 1 cubic centimeter

of the diluted mixtures and the number of units absorbed per cubic centimeter by 1 oese of the organism are as follows:

Dilution of the serum to which the bacte- ria were	Original value of 1 cubic cen- timeter of the dilut- ed serum.	Value of 1 cubic centimeter of the diluted serum after the absorption of 1 oese per cubic centimeter of—		Number of units absorbed per cubic centimeter by 1 oese of—	
added.		Virulent.	Avirulent.	Virulent.	Avirulent.
1-20 1-100 1-500	1, 200 240 48	50 12 10	1, 120 225 45	1, 150 228 38	80 15 3

¹ In units of immunity.

From this table (and Table X) we see that in the dilution of 1:20 the virulent organism had bound about fourteen times as many amboceptors as the avirulent one $(\frac{230}{240}$ to $\frac{16}{240})$; and in the dilution of 1:100 about fifteen times as many $(\frac{228}{240}$ to $\frac{15}{240})$. However, it must be mentioned (and as may be seen from Table X) that the value of the serum in the dilution of 1:20 after treatment with the virulent organism, varied with different guinea pigs between 1:900 and 1:1,000, and after treatment with the avirulent between 1:22,000 and 1:22,400. If we regard the lower value in each instance as the correct one, then the ratio of absorption must be considered as $\frac{231}{240}$ to $\frac{20}{240}$, or as 11:1. but if the higher values are regarded as correct the ratio is as $\frac{230}{240}$ to $\frac{16}{240}$, or as 14.1.

In the dilution of 1:500 the serum, after treatment with the virulent organism, showed a value of 1:5,000 (0.2 milligram); while that previously treated with the avirulent one showed a value of 1:22,500 (nearly 0.04 milligram). Therefore the value of the latter serum was less than five times as great as that of the former, the ratio of absorption being $\frac{15}{240}$ to $\frac{190}{240}$, or about 12:1. However, the experiments with the serum dilution of 1:500 might be misleading without the following explanation.

As already pointed out, the agglutination of the virulent organism in the dilution of the serum of 1:500 was not complete, even after the mixture had stood for two hours at the temperature of the ice box, and after prolonged centrifuging the overlying fluid was still not entirely clear. Evidently there still remained in this supernatant fluid above the precipitate a few vibrios in which a suf-

¹With only one animal was this value as low as 1:20,000.

ficient number of the haptophore or agglutinophore groups of the agglutinable substances necessary to bring about the phenomena of agglutination were not yet bound by their respective groups of the agglutinin. When this serum was injected into guinea pigs it clearly carried with it not only the free bacteriolytic amboceptors of the serum but also those bound to the bacteria and remaining in the slightly cloudy fluid. These amboceptors, meeting with a suitable complement in the abdominal cavity of the guinea pig (and combined with an additional number of amboceptors if necessary), obviously destroyed the bacteria to which they were already united by their haptophore groups before inoculation; and, in this manner (the amboceptors), being set free, were capable of again unfolding their bacteriolytic action against one oese of the fresh living organisms introduced for the regular bacteriolytic test. Hence, owing to the combined action, within the abdominal cavity of the guinea pig, of the free amboceptors in the serum introduced and of those carried in with it bound to the bacteria, the value of the serum appeared higher than it would have done if the agglutination of the organisms by it had been complete and they had been in this manner originally separated from the mixture.

In support of this argument it may be seen from the experiments shown in Table X that, while in the dilution of the serum of 1:500 after treatment with the virulent strain (where agglutination was not complete), we find a bactericidal value of 0.2 milligram (1:5,000), in the dilution of 1:100, after treatment with the virulent organism (where agglutination was originally complete) we find a value of only 0.8 milligram (1:1,200).

The actual bactericidal value of this serum (dilution of 1:500) in which the agglutination of the virulent organism was not complete could not be accurately determined after the removal of the incompletely agglutinated bacteria by filtration, since in this process, while the combined amboceptors were separated, a portion of those unbound also remained behind on the filter.

In this same dilution of the serum (1:500) with the avirulent organism, the agglutination of the spirilla being complete on account of the fewer agglutinable receptors necessary to be occupied in order to bring about this phenomenon, no bacteria, binding amboceptors, were carried into the abdominal cavity of the guinea pig, and the value of the serum was practically the same (viz, 1:22,500) as in the lower dilutions of 1:20 and 1:100.

That the explanation thus given is applicable to these results is confirmed by the very important research of R. Pfeiffer and Friedberger (16), published, however, several months after the experiments mentioned above were completed. These authors show conclusively that the cholera amboceptors bound to cholera spirilla were not destroyed, either in the event of the death of the organisms (bacteriolysis) or in that of their subsequent life, but that they were in both instances eventually set free from the bacteria and again became capable of exercising their bacteriolytic power. A catalytic action is therefore suggested.

On comparing the results of Tables IX and X, emphasis is again laid on the fact that in the cholera-immune serum of both rabbits and guinea pigs the virulent organism usually bound from eleven to seventeen times as many bacteriolytic amboceptors as the avirulent one. This difference in the power of binding practically corresponds to the difference in virulence between our two strains, "virulent" and avirulent." Hence these experiments bear out the hypothesis that the virulence of a living cholera organism is proportional to the number or degree of affinity of its bacteriolytic haptophore groups.

Another point which is demonstrated by this investigation is that the organisms of each strain bind proportionately the same number of amboceptors in the rabbit cholera-immune serum as in that of the guinea pig. This suggests that the cholera amboceptors of rabbits and guinea pigs unite to the same receptors of the cholera vibrio—that is, that the receptors of this organism are identical for both animals. Pfeiffer and-Friedberger (16), since these experiments were performed, have shown by an entirely different method of experimentation that the receptors of the cholera spirillum are identical also for goats and rabbits.

The avirulent organism was next passed successively through the abdominal cavities of about twelve guinea pigs and then examined in regard to its virulence. This was found to have considerably increased, since now three-fourths oese of the organism produced death in a guinea pig of 250 grams' weight within twenty-four hours. Unfortunately, at this time there was no longer on hand any serum from either of the animals with which the series of experiments given in Tables IX and X were performed, so that a compara-

¹A brief summary of the results of my experiments was published in American Medicine, Vol. VI, August 15, 1903.

tive study could not be made. However, with another rabbit choleraimmune serum, to which were added corresponding amounts, first, of the original avirulent strain (of 1½ oesen virulence), and, secondly, of the avirulent strain after about twelve successive passages through guinea pigs (of three-fourths oese virulence), it was found that the latter strain was able to bind in the immune serum nearly twice as many amboceptors as the former. In other words, with an increase in the virulence of the organism, an increase in the number or binding power of its haptophore groups had occurred.

It would be interesting to follow this relationship quantatively to its logical conclusion, and to plot the result as a curve. In this way a mathematical basis of the relation between receptors and amboceptors might be obtained and light thrown on the nature of this relation. This work will be shortly undertaken in this laboratory.

COMPARISON OF THE AGGLUTINATION OF THE VIRULENT AND AVIRULENT STRAIN.

In the work on protective inoculation against cholera, already referred to, it became evident that the avirulent organism was agglutinated by higher dilution of the same sera than the virulent one. This may be seen in Tables I to VIII of this article. However, it is true that sometimes the difference in agglutination varied, owing to the fact that the serum used was not always of the same age; and in case varying amounts of agglutinoid were present, the usual ratio of agglutination between the two stems was lost, because smaller amounts of agglutinoid prevented agglutination from appearing in suspensions of the avirulent than in suspensions of the virulent organism. Theoretically these results could be explained on the assumption that there existed fewer agglutinable haptophore groups in the avirulent than in the virulent strain, and hence a smaller number of uniceptors was necessary to bring about a reaction in the case of the avirulent germ than in that of the virulent one, so that with the former organism agglutination took place in higher dilutions. Likewise when sufficient agglutinoid was present smaller numbers of such modified uniceptors would suffice to bind the receptors of the avirulent strain than would be required by the virulent one. Hence the phenomenon of agglutination would fail with the avirulent organism in lower dilutions than it would in the case of the virulent one. However, even in a fresh cholera-immune serum no such difference as 15:1 could ever be demonstrated in the agglutinable power of the two stemsthat is, the avirulent strain was not agglutinated in dilutions fifteen times higher than those necessary in the case of the virulent race. Neither could it be shown in the same sera (used in the experiments of Table X) that the virulent strain bound fifteen times more agglutinin than the avirulent, though it was true that the amount of agglutinin which the former organism appropriated was about four or five times as great. We may then argue that, while the virulent organism contains more agglutinable substance than the avirulent—that is, that its agglutinable haptophore groups are more numerous—its virulence (from an infectious point of view) is not in direct proportion to the amount of such substance, and just as the amount of agglutinin in a serum has not been found to be directly proportional to the degree of (at least the bactericidal) immunity of the host, so it may now be stated that neither is the amount of agglutinable substance directly proportional to the (infectious) virulence of the organism. Obviously we must not lose sight of the fact that, just as in the immunity of the host, two, and perhaps three, factors may enter into consideration, namely, the antitoxine, the bacteriolysine, and the agglutinine, so, in the question of the virulence of a cholera organism, there may also be three substances to be considered, namely, the toxine, the bacterial cell, and the agglutinable substance.

From our study as to what properties the virulence of our two cholera stems depends upon, and from our recorded animal experiments, it seems that it is the power of the cell to bind bacteriolytic amboceptors and to resist destruction (bacteriolysis) as well which is the most important element connected with the death or recovery of the animal (our indicator of the virulence), and that it is the ratio of this power (both to bind and at the same time to prevent bacteriolysis) rather than that of any other which we express, when we state that the virulence of the organisms is as 15:1. Therefore, even should the agglutination proceed in a manner parallel to that of the bacteriolysis, it is questionable whether we should expect, a priori, that our virulent organism would bind in the immune serum fifteen times more agglutinin than the avirulent; since it has not been demonstrated (even granting for the moment that the amount of agglutinable substance which the organism possesses is a possible preliminary factor entering into the question of the virulence) that the ratio of the amounts of the agglutinable substance contained in the two stems is as 15:1. Indeed, in so

far as the amount of this substance could be demonstrated from the action of the strains upon an immune serum, the avirulent organism was never agglutinated in dilutions higher than about five times those which agglutinated the virulent strain. However, Eisenberg and Volk (17) thought that it was doubtful whether the agglutinable substances of an organism could be fully saturated, since they were able to find almost no limit to this power. Moreover, it is well known that the more concentrated the serum in agglutinine, the greater is the quantity of agglutinine bound by the same amount of agglutinable substance. The question of the velocity of the reaction should, therefore, be carefully considered in relation to this phenomenon.

In connection with this subject, the very recent work of Arrhenius (18) is also of interest. This author found that for constant quantities of bacteria, in which equation the amount of free agglutinine = B and the amount of bound agglutinine = C, the following relation exists: $C = Konst. B_3^2$ However, if the quantity of bacteria (A) varied, the following equation was found to exist, viz, $\frac{C}{A} = KB_3^2$. That is, the absolute quantity of bound agglutinine did not enter into the question, but only its concentration in its solvent—the bacteria. He pointed out that, with a knowledge of the conditions of equilibrium the assumption that the agglutinine exists as a number of substances with binding properties of different degrees of affinity is superfluous, even if the possibility that the agglutinine is a mixture of several active bodies can not be denied.

Arrheninus apparently worked with a single strain of an organism of a certain virulence. It would be very interesting to perform these same experiments with strains of different virulence and with the free agglutinable receptors of such organisms. We might expect, a priori, that the same law would apply in such experiments, since by the "quantity" of the bacteria we probably really understand the number of agglutinable haptophore groups, which, in the case of the virulent organism, would be greater than in that of the less virulent strain. Hence $\frac{C}{A}$ would vary with the virulence of the organism.

However, we must return to the question of the virulence of the two strains of cholera spirilla and defer for the present any further discussion of this matter.

ABSORPTION EXPERIMENTS WITH THE KILLED ORGAN-ISMS AND RABBITS' IMMUNE SERUM.

With the hope of throwing more light on this relation, a study of the effect of the absorption of the amboceptors from the same immune serum by means of the killed organisms was undertaken. The minimal lethal dose for guinea pigs of the spirilla killed with chloroform was first carefully determined for each strain. It was found that about 5 or 6 oesen of the killed virulent organism, when introduced into the abdominal cavity, produced death within twenty-four hours in guinea pigs of 250 grams' weight, while about 8 or 9 oesen of the killed avirulent strain were necessary to cause the same result. Therefore, with the killed organisms a ratio of virulence of nearly 2:1 existed. These results speak partly in favor of the hypothesis of Gotschlich and Weigang (6), though from them it is evident that if the virulence of a living organism depended only upon its power to multiply more or less rapidly within a given time, the virulence of our two strains of cholera spirilla (provided that exactly the same amounts of each were used) would probably be equal after the death of the organisms. However, this, as we have just seen, is not the case, a difference in virulence of nearly 2:1 existing between the two killed races. On the other hand, in the living state a difference of virulence between the organisms of 15:1 existed. Therefore, these experiments would support the idea that the energy of growth of an organism is a factor of importance, though not the only one, in relation to its toxic virulence.

For the absorption experiments with the killed organisms the same rabbit cholera-immune serum was used as was employed for those recorded in Table X with the living spirilla.

Five oesen of each strain, the virulent and the avirulent, were suspended in 5 cubic centimeters of bouillon in separate test tubes,

^{&#}x27;It may be justly argued that this is merely a toxic ratio and not one of virulence. However, if we assume that the toxine is intracellular, the binding power of each strain of the killed organisms for bacteriolytic amboceptors, as will be pointed out further on, is apparently of some importance in the liberation of this toxine, and since the bacteriolytic receptors are partially concerned in this liberation, it has been thought advisible to employ the term virulence (in this connection) as representing the injurious influence of the organism upon a susceptible being (the guinea pig). It is admitted that ordinarily the terms virulence and toxicity may with advantage be distinguished from one another.

to each of which were then added fifty drops of chloroform. After the organisms had been killed, the chloroform was evaporated, the sterility of the mixtures demonstrated, and then there were added to each of the two tubes 5 cubic centimeters of the rabbit serum in dilutions of 1:20. Hence the dilutions of the serum in each of the two tubes containing 5 oesen of the organisms equalled 1:40. The mixtures were allowed to stand for two hours, and after complete agglutination had occurred in both of them, the clear fluid above was pipetted off and examined in each instance for its bactericidal value. The results of these experiments, calculated for 1 cubic centimeter of the undiluted serum, may be seen in Table XI. We first notice that the receptors of the organism have been seriously injured or diminished through the killing of the spirilla by chloroform; for, whereas the living virulent organism bound about $\frac{23}{24}$ of the bactericidal amboceptors in the same rabbit serum (see Table X), the killed virulent organism was able to bind only about $\frac{19}{24}$ of them. This loss or injury of the receptors apparently progresses to a certain extent with the loss of virulence, the minimal lethal dose of the virulent strain being for the killed organism about 5 or 6 oesen and for the living one $\frac{1}{10}$ oese. However, while the ratio of virulence between the killed and the living germ may be expressed as about 1:55, the difference in their power to absorb the bacteriolytic amboceptors is not at all in this proportion. Hence it would appear that the relation between the virulence of the killed organism and that of the living one, both belonging to the same strain, is not always, at least, dependent upon the number or binding power of the bacteriolytic haptophore groups possessed by each, or at any rate not dependent alone upon this condition. In other words, it would seem that in the death of the bacteria by the process described a certain change has taken place in the organism, so that while the receptors may be able to bind in vitro a considerable number of amboceptors, the poisoning action of the bacteria in the animal body is not unfolded to the extent which might be expected. Therefore the idea is suggested that in the killing of the organism with chloroform the intracellular toxine has also suffered a change—that is, it now has been placed in such a condition as not

¹In making this comparison, we must assume that the living bacteria in the experiments of Table X had not multiplied during the time that they were in contact with the serum (two hours) at the temperature of the ice box.

to be so easily set free from the bacteria (possibly through a retarding of the action of the ferments of the organism), or it has actually been altered chemically. In other words, in the case of the killed bacterium entirely another factor besides its power of binding amboceptors and its resistance to destruction (bacteriolysis) would seem to enter principally into the question of virulence (or the fate of the inoculated animal), namely, the condition and the amount of the intracellular toxine set free.

We have seen that with the killed virulent organism amounts as large as 5 or 6 oesen are necessary to bring about the death of a guinea pig. On the other hand, it has been demonstrated by the experiments in which the killed organisms were added to the immune serum, in vitro, that the killed bacteria still contained a considerable number of haptophore groups, as the proportion of the amboceptors removed from the serum demonstrates. Indeed, they were capable of binding many more amboceptors than would be necessary in order to bring about their complete dissolution in the animal body. Hence upon their introduction into the abdominal cavity of an animal, probably the factor of the greatest importance in relation to its death or recovery would be the condition and the amount of the intracellular toxine which the bacteria contained. The virulence in this instance would chiefly depend upon this value.

Possibly the slight and partial injury by chloroform of the agglutinophore group of the agglutinable substance in the dead bacteria may also be a preliminary factor which exercises a retarding effect upon the virulence, by placing the organism in a less satisfactory condition for the liberation of the toxine.

After a comparison of the values of the sera following treatment, first with the living, and then with the killed avirulent organism (see Tables X and XI), we are inclined to the same explanation, though some of the results can not be satisfactorily interpreted. Thus it must be stated here that even when a smaller number of receptors (than was contained in five oesen of the avirulent organism) was placed in this immune serum it was found afterwards that generally a loss of amboceptors of from about 0.001 to 0.003 milligram per cubic centimeter had occurred in the undiluted serum—that is, when the number of receptors existing in the avirulent organism became very small, they seemed endowed, upon

being placed in a concentrated serum, with a slightly greater binding power. Thus, in this series of experiments, with the avirulent organism the killed germ absorbed apparently the same number of amboceptors as the living one, though in each case this was actually very small. However the ratio of virulence of the living germ to the killed one was about $5\frac{1}{2}:1$ ($1\frac{1}{2}$ to 8 or 9 oesen).

On comparing the value of the serum added to the killed virulent organism with that of the one added to the killed avirulent organism, we see that the former has about one-fourth the strength of the latter; or, that the virulent organism has bound $\frac{19}{24}$ and the avirulent one $\frac{2}{24}$ of the bacteriolytic amboceptors of the serum. Beginning, then, with a ratio of virulence of about 2:1, we obtained an absorption ratio of (bacteriolytic) amboceptors of about 9:1—that is, the virulent organism bound nine times as many amboceptors as the avirulent one. However these results are not confusing since it has already been pointed out that the ratio of 2:1 is mainly a ratio of the toxic haptophore groups, while that of 9:1 is a ratio of the bacterial haptophore groups of the two strains. But as has been pointed out above, even when such an absorption of the amboceptors by these respective organisms occurs in the animal body, an absorption which is evidently far greater than that necessary for the complete dissolution of the bacteria and the liberation of the toxine, the death of guinea pigs will not result with smaller doses than 5 or 6 oesen of the killed virulent organism, and 8 or 9 oesen of the killed avirulent one, for the reason that in smaller amounts of the bacteria (killed after this manner) there is not present a sufficient amount of the unchanged toxine to accomplish this end. This once more forces us to the conclusion that the difference in virulence between the organisms killed with chloroform in this manner is not alone dependent upon the number or the binding power of the bacteriolytic haptophore groups, but also upon the number and binding power of the toxic haptophore groups—that is, the amount and the condition of the intracellular toxine present in the organism at the time of its inoculation.

On the other hand, the virulence of the killed organism may depend to a certain extent upon the number or the avidity of the bacteriolytic haptophore groups, since the greater the number present or the greater their binding power, the larger the quantity of amboceptors excited and then bound (within a given time), and hence, the quicker the complete dissolution of the bacteria and the greater the amount of toxine liberated within a given moment, and therefore the greater the injury to the animal. We have already seen that in the killed virulent strain the bacteriolytic haptophore groups are actually much more numerous or endowed with much greater binding power than in the killed avirulent one, but in this connection it must again be noted that it is not a question of the bacteria being killed by the amboceptors (death has already taken place) but it is their dissolution which we suppose results with the virulent strain within a shorter period of time. Were we considering the living bacteria, the hypothesis would necessarily be somewhat different.

The living virulent organism evidently has greater powers of resistance than the avirulent, and more amboceptors are required for its destruction, but through the possession of an increased binding power in its haptophore groups, and hence its greater avidity for amboceptors, it is more capable both of appropriating and of giving rise to the production of these groups in the animal body than is the avirulent germ. Therefore, upon the entrance of the virulent bacteria into a susceptible individual a number of the organisms become more quickly destroyed by means of this power to absorb whatever amboceptors are present or are produced at the time of their introduction. The intracellular toxine of these organisms is thus liberated. However, since a sufficient number of amboceptors to satisfy the avidity of all the organisms is not at once produced, the remaining living bacteria during this latent period multiply rapidly through the increased energy of growth which the virulent organism possesses. As soon as the animal body has responded to an additional production of amboceptors or a sufficient number are set free from the bacteria which have already been killed, an additional number of the virulent organisms are bound and destroyed, and a fresh intoxication of the host results; Hence, the virulence of the living cholera spirillum depends, probably, both upon its power of resistance to the amboceptors and its power to excite and to absorb these substances as well as upon the amount of intracellular toxine (the number of toxic haptophore groups) it possesses and its energy of growth. In this connection it is well to call attention to the work of Von Dungern (19) who concluded, from a series of inoculations in animals, that the virulence of two strains of cholera spirilla was independent of their toxic properties. However, it hardly seems that one would be justified in drawing such a conclusion from Von Dungern's experiments. It would appear, at least from his results with the intraperitoneal inoculation of guinea pigs with the killed organisms, that the virulent organism was more toxic than the less virulent one, though it is true that there was certainly a great difference in the ratio of virulence when compared with the ratio of toxicity, the latter being nearly identical. These results, however, practically coincide with our own, namely, that with a difference of virulence of 15:1 with the living organisms, we obtained a toxic ratio with the dead strains of less than 2:1.

It now seemed desirable to study the effect upon this immune serum of the free bacterial receptors of the cholera spirillum in solution, obtained by autolytic digestion and prepared both from the virulent and the avirulent strain.

ABSORPTION EXPERIMENTS WITH THE FREE RECEPTORS OF THE ORGANISMS AND RABBIT'S IMMUNE SERUM.

Accordingly, 5 cubic centimeters of the virulent and 5 cubic centimeters of the avirulent cholera prophylactic were each mixed separately with 5 cubic centimeters in dilutions of 1:20 of the same rabbit immune serum employed in the experiments comprising Tables X and XI. After allowing the mixtures to stand for two hours, only a very faint precipitate had taken place, though the fluid above became slightly cloudy in both of the tubes, and more so in the one treated with the avirulent prophylactic than in the other. Prolonged centrifuging did not clear the overlying liquid; and in this condition it was pipetted off from the two tubes and examined separately for its bactericidal properties. (See Table No. XII.)

The serum after treatment with the virulent prophylactic showed a value (calculated for 1 cubic centimeter of the undiluted serum of about 0.07 milligram (1:14,000), while that treated with the avirulent one showed a value of about 0.04 milligram (1:23,000). The virulent prophylactic had apparently absorbed about $\frac{1}{24}$ of the amboceptors present and the avirulent one somewhat less than $\frac{1}{24}$, a ratio of absorption of about 10:1. However, these results must be regarded with caution, since we were here probably encountering conditions much the same as those seen in the experiments performed

with the living organism in the higher dilutions of the serum; that is, the bacteriolytic amboceptors united to the receptors were present in suspension in the slightly cloudy mixtures. Upon injection of this serum into animals, these amboceptors, after the destruction or solution of the receptors through the aid of the guinea pig's complement (or of more amboceptors), were not destroyed but again set free; and once more uniting with the receptors of the freshly introduced bacteria evidently gave to the newly added serum an apparently higher bactericidal power. Therefore, the value of the serum after treatment with the virulent prophylactic is probably actually somewhat lower than 0.07 milligram (1:14,000); and the same may probably be said of the value of 0.04 milligram (1:23,000) of the serum after treatment with the avirulent strain; although the precipitation of the receptors was probably more complete in this instance than in the case of the virulent prophylactic. Furthermore, in the higher dilutions a smaller number of the combined amboceptors was obviously carried into the animal body. It was impossible to obtain accurate results upon the filtration of the fluids, through very dense filters, for, while this process removed the combined amboceptors and receptors, it also removed, as was shown by a control experiment made with the immune serum alone, a considerable number of the free amboceptors. The more concentrated the serum the greater the number of amboceptors removed by filtration. On the other hand a coarser filtration with filter paper did not separate the combined receptors in suspension. Therefore, this series of experiments suggests forcibly (although it does not conclusively demonstrate) that the binding power of the two prophylactics (that is, of the free receptors) added to an immune serum is within certain limits proportional, first, to the immunity caused by each after its injection into animals (see Tables III-VII), and second, to the infectious virulence of the respective strain from which it is prepared.

COMPARISON OF THE IMMUNITY OBTAINED WITH THE FREE RECEPTORS OF THE VIRULENT AND AVIRULENT STRAINS.

We may now compare the immunity produced by the injection into rabbits of the free receptors of the virulent cholera organism with that produced by the injection of those of the avirulent one. These free receptors were obtained, as has already been stated, by the

filtration of the killed cholera organisms, which had been subjected to autolytic digestion for varying periods of time in aqueous solutions. These free receptors in the fluid constitute the cholera prophylactic. The strength of the prophylactic varies in the different series of experiments according to the number of oesen of the bacteria digested in each cubic centimeter of the fluid. In prophylactic No I (see Table No. III) 1 cubic centimeter contains the number of receptors obtained by the digestion of one oese of the killed organisms. The strength of the prophylactic in the other series of experiments is indicated in Tables IV-VII, where the results in immunity are also shown; the tables are self-explanatory. In the animals of Table VII and in a portion of those of Table VIII the inoculations were made subcutaneously, the rabbits comprising the former receiving injections of the prophylactic in liquid form, and those of the latter the same substance dried in a vacuum and redissolved in saline solution. On comparing the immunity obtained by the injection of the virulent and avirulent receptors from Table III we see that the ratio of bactericidal immunity between the animals inoculated with the virulent and those inoculated with the avirulent prophylactic varies between 3.5:1 and 12:1. In Table V the sera obtained from the animals inoculated with the virulent prophylactic showed a bactericidal value of about five and one-half to twelve times that obtained from the injection of corresponding amounts of the "avirulent," and in Table VI the animals of the "virulent" series developed sera having six to fifteen times the value of those of the "avirulent" one. In Table VII, with subcutaneous inoculation (Nos. 399, 400, 423, 184), the proportion is from 1:8 to 1:11 and in Table VIII, with the dried prophylactic, the relation is from 1:1.33 to 1:4. The results obtained with the dried prophylactic are certainly not as accurate as those given by the fluid, because of the manipulations to which the powder was subjected; and since they are not in agreement with all of the other numerous experiments, in which the liquid prophylactic was employed, for the purposes of this argument they must be discarded. With this exception the results here reported with the free receptors are in accord with those which have been obtained by other observers who for inoculation have employed strains of the killed organisms of different virulence; namely, that the immunity obtained is within certain limits approximately proportional to the virulence of the inoculated virus.

COMPARISON OF THE IMMUNITY OBTAINED WITH THE LIVING VIRULENT AND AVIRULENT STRAINS.

We will next turn our attention to the results in immunity obtained by the inoculation of the living virulent and avirulent cholera spirilla. In these experiments the rabbits were given intravenously one-half oese of the living organisms of the respective strains suspended in 1 cubic centimeter of bouillon. Two series of six rabbits each were inoculated, and on the day of the operation in each instance the ratio of virulence between the two strains was verified as 15:1. The results are recorded in detail in Tables I and II, from which we see that by the intravenous injection of the living organisms in quantities of one-half oese the ratio representing the bactericidal value of the sera of the animals inoculated with the virulent and the avirulent organisms was never greater than 4.5:1—that is, the virulent organism never furnished a serum more than four and one-half times as potent as the avirulent one. Therefore, it can not be said that the immunity obtained was directly proportional to the virulence of the organisms, since the latter was 15:1 before inoculation. However, with the digested extracts of the organisms of different strains, as we have just seen, and the killed organisms of different degrees of virulence, this may, within certain limits, be said to be the case.

How shall we explain this discrepancy between the virulence of the living organisms and the immunity produced by each? It may be argued that in such a complicated process as immunization the animal cells could not be expected to respond in a proportional manner to such great differences in stimuli, and further that with such doses, as large as one-half oese of the organisms, we could not expect the immunity to increase proportionately to the virulence, since the animal cells are capable of responding only to a certain limit in the production of immunity, no matter how great the stimulus, and since the number of amboceptors given off becomes proportionally (to a given stimulus) less and less as one approaches this limit. If one is convinced by such an argument, for which it is true there is considerable supporting evidence, no further explanation is necessary. However, on the other hand, it may be seen from the experiments with the intravenous inoculations of the free bacteriolytic receptors from both strains (see Table VI), where the receptors from 12 oesen of the organisms were injected, that the serum of the rabbits inoculated with the receptors from the virulent strain showed a value from six to fifteen times as great as that of those treated with receptors of the avirulent organism.

In these experiments it is to be noted that the stimulus from the receptors of 12 oesen of the virulent strain was equally as great as that from one-half oese of the living virulent organisms, as is evidenced by the fact that about the same bactericidal immunity was obtained in the sera of the animals treated with the virulent strain (comprising Tables I and VI); however, it must be observed from the experiments of Table V, in which more receptors were evidently obtained upon a more complete digestion of the organisms, and where the stimulus from the amount of receptors was evidently stronger than that from one-half oese of the living virulent organisms, as shown by the value of the sera obtained, the ratio of immunity fell in one instance as low as 5.5:1. Yet in the other the ratio stood at 12:1. So throughout these experiments (see Tables III to VII), made to determine the comparative value of the sera of more than twenty rabbits, the animals inoculated with the receptors of the virulent strain furnished a serum from three and one-half to fifteen times as strong as that from the animals inoculated with a corresponding amount of those from the avirulent one. However, in only one instance was the low ratio of 3.5:1 obtained, the next lowest being 5.5:1, and the next 6:1 and 8:1.

Likewise, turning to results other than our own, we see that Ascher (20) found, upon the intravenous injection of varying amounts of the killed cholera spirillum into rabbits that 1 oese gave rise to a bactericidal immunity more than thirty times as great as one produced with one-tenth or two-tenths oese. He also observed that, while 2½ oesen in two cases gave less than twice as great a bactericidal immunity as 1 oese, 10 oesen produced a serum of ten times the bactericidal power of the one produced by 1 oese. Therefore, while evidently the individuality of the animal is an important factor in the degree of the immunity produced, a fact borne out by the varying results seen in our Tables, and while also it is evident that with very large doses the immunity is not directly proportional to the quantity of the organism inoculated, at least for amounts of one-half oese (reasoning from the immunity obtained with the free bacteriolytic receptors and that with the killed organisms) the explanation of the difference of immunity of 4:1 as

against that virulence of 15:1 is still lacking. At least no explanation given previously would seem to be satisfactory, whether it be solely upon the ground that the cells in the case of the animal inoculated with the virulent strain have already produced the maximum amount of amboceptors of which they are capable, or upon the ground that their limit of production of amboceptors is so nearly reached by a stimulus resulting from a considerably smaller number of receptors thon one-half oese of the virulent organism furnishes, that the increased stimulus produced by this amount of the virulent organism gives rise to so small an increase in immunity that its ratio to that produced by one-half oese of the avirulent strain is never greater than 4:1.

Therefore, while it is admitted that we should not necessarily always expect in our animals an immunity fifteen times as great from the injection of a stimulus fifteen times as powerful, we might anticipate, if we reason from the results obtained by the injection of the killed organisms or their extracts, that a ratio of immunity nearer to 15:1 than that of 2.5:1 to 4.5:1 would be obtained, when amounts not larger than one-half oese of the living organisms are injected. Hence it would seem necessary to seek for some other explanation for these results. The idea is suggested that something has happened to the living avirulent strain after its injection into the animal which increases its virulence and brings it into greater similarity with the virulent one, so that the ratio of 15:1 is lessened; the dissimilarity being existent at the moment preceding injection as is evidenced by the fact that the virulent organism will kill in doses one-fifteenth as great as the avirulent. Should the different strains be killed at this moment and injected, or killed and digested and then injected, this change does not take place. The ratio of 15:1, as evidenced by the immunity obtained, is within certain proportions retained, hence depriving the avirulent strain of its life would seem to be at least one of the factors which prevented this change in the ratio.

Let us now consider the influence which an animal which has succumbed to an infection with a given bacterium has exerted upon the infecting organism during the period of its parasitic life upon the host, and also the influence which a normal immune serum exerts upon the virulence of a bacterium which has been cultivated in it.

Since the classical observations of Pasteur and his pupils in 1881, (21) we have known that in general attenuated races of bacteria can be reëendowed with lethal properties by successive passages through susceptible animals. Indeed, this is the method usually employed for increasing the virulence of a given bacterium. However there is a limit to this with every organism, and a culture which had attained its highest possible virulence was designated by Pasteur as a "fixed virus" (22).

We also know from many observations that the virulence of an organism may be greatly increased by its repeated inoculation into fresh serum. Thus Roger (23) as early as 1889 reported that streptococci which through cultivation in bouillon had lost most of their energy of growth and virulence would regain these powers when they were repeatedly inoculated in rabbit serum.

Trommsdorf (24) also found that organisms which had been grown in fresh serum showed an increased resistance to bacteriolysis. Dansyz (52) maintained that anthrax bacilli, when inoculated into fresh serum, became surrounded with a sort of mucous covering, which later protected them, to a certain extent, against the action of bactericidal serum.

Metchinhoff and Roux (26) have shown that the virulence of an organism may be greatly increased by growing it in collodion sacs within the abdominal cavity of an animal.

Professor Welch, (27) in his Huxley lecture on recent studies in immunity, in advancing an hypothesis by which might be explained the source, the mode of production, and the nature of certain bacterial toxines, pointed out that "certain substances of the host of cellular origin assimilable by the parasites through the possession of haptophore groups with the proper affinities become anchored to the receptors of the parasitic cell, which, if not too much damaged, is thereby stimulated to the overproduction of like receptors. These excessive receptors of the parasite, if cast into the fluids or cells of the host, are constituted intermediary bodies or amboceptors with special affinities for these cellular constituents or derivatives of the host, which many lead to their production and for which they possess in whole or in part identical receptors. Provided the host is supplied also with its appropriate complements, there result cytotoxines with special affinities for certain definite cells or substances of cellular origin in the host. The contribution of the parasitic cell to these cytotoxines is the

amboceptors; either the parasite or the host may provide the complements."

In considering the condition of the bacterium as well as that of the animal host, according to the hypothesis advanced, the struggle between the bacteria and the body cells in infections may be conceived as an immunizing contest, in which each participant is stimulated by its opponent to the production of cytotoxines hostile to the other and thereby endeavors to make itself immune against its antagonist.

Ainley Walker (28) performed a series of experiments which, so far as they went, supported this hypothesis of Professor Welch. Walker showed that by growing typhoid bacilli in bouillon to which were added increasing amounts of immmune serum (free from the complement), that their virulence and resistance to serum were increased and their agglutinability diminished. In another series of experiments, (29) he found that the progressive passage of typhoid bacilli through fresh bacteriolytic normal rabbit serum mixed with bouillin in the proportion of 1:10 produced a distinct increase in the virulence of the bacilli toward rabbits and guinea pigs, and also increased their resistence to bacteriolytic serum, as shown by the plate culture method.

Welch's hypothesis includes the explanation which Walker gives for the results of his experiments, and also more. According to the idea of the former, certain bacterial antibodies (discharged receptors) are capable, not only of neutralizing the immune bodies of the host, but with aid of the complements also of poisoning the cells of the latter.

Keeping these ideas in view, let us attempt next to trace the biology of an avirulent strain from the moment of its intravenous injection into a nonimmunized rabbit. Upon the arrival of the organisms in the blood stream they quickly disappear and indeed are, we suppose, soon killed, though just how rapidly we do not know. Pfeiffer and Marx (30) assumed that in a short time the cholera spirilla became anchored to the cells of the spleen, the bone marrow, and the lymph glands, since it was in these organs that the specific protective substances were particularly formed. However, in a later series of experiments Pfeiffer (31) was not able to demonstrate conclusively this increased anchoring power for cholera spirilla of the cells of the spleen.

It would seem to be a mistaken idea to suppose that the

immediate destruction of the organisms is in all cases inevitable, since we know, for example, from the injection of avirulent strains in *micrococcus melitensis* into the blood current of monkeys, that the organisms may remain alive for a period of time and then be reobtained in cultures. Yet eventually they disappear and the recovery of the animal results.

We also know that in many infectious diseases (typhoid fever, etc.) the organisms may be isolated by culture from the circulating blood, and yet finally these bacteria become destroyed and the patient survives the malady.

The most important results in this connection which have been obtained with the cholera spirilla are those of Kolle (32). He found that upon injection of one-half oese of the living cholera organisms into the carotid of guinea pigs, that blood drawn at intervals of from five minutes to fifteen minutes after the injection contained but few living organisms since when it was inoculated in streaks upon an agar plate only a few and widely scattered colonies developed upon the media. Evidently within this time the majority of the organisms had been destroyed, although some were still alive. In these experiments and at the same period of time, the bacteria were found to be no more numerous in the spleen than in the blood current. From the observation of Pfeiffer's phenomenon in the abdominal cavity of guinea pigs, we also know that in some cases (even when the animal eventually recovers) some of the organisms may remain alive for more than one-half hour after their injection, and it is only later that they become disintegrated.

For the moment then, let us suppose that our organism is of a sufficiently great virulence to be capable of surviving for at least a few generations, and that, while the animal becomes very ill after inoculation, it eventually recovers. These successive generations of the bacterium multiplying in the rabbit serum will, we suppose, rapidly increase in virulence—that is, their haptophore groups will rapidly rise in number, owing to the stimulus received from the occupation of the receptors of the bacteria by the amboceptors of the normal serum. (Compare with Walker's results with typhoid bacilli in fresh normal serum.) That is, reasoning from the well-known biological law of Weigert, an injury to the bacterial cells will be produced and an excessive generation of the receptors result. Hence the final results in immunity in this instance will be much greater than it would if

the successive generations became no richer in receptors than the one existing at the time of inoculation.

On the other hand, let us trace the fate of the virulent organism upon its injection into the circulation. This strain has already reached its maximum virulence and become a "fixed virus"—that is, it is already saturated with haptophore groups. Therefore its few successive generations can become no richer in such groups than the one used for the inoculation, so that the immunity produced can only correspond to, or at least only equal, that which would result from the generation existing at the time of inoculation, multiplied by the number of generations for which the organism survives. Therefore the immunity obtained by the organism of maximum virulence would not be so great, compared with the stimulus, as would that produced in the case of the living avirulent germ. Furthermore, is it not conceivable that if the same stimulus were received by the virulent organism as by the avirulent one, the latter, which is so poor in receptors, would feel the injury more severely than the former, which is so well protected and so rich in these bodies? Hence would not the regeneration, provided an immediate destruction of the organism did not occur, eventually be greater in the case of the avirulent strain? We know that the number of receptors in the virulent organism must be enormous. We can conceive that it may possess many more receptors than would be required to bind all the existing amboceptors in the normal serum. Therefore if there is still an excess of unbound receptors, will this organism be stimulated as strongly to the generation of others as the avirulent one in which no such excess exists?

Such theoretical conceptions are difficult to confirm. In the first place, it does not seem likely that even one division of the cholera spirillum would take place after its inoculation into the blood circulation, since the shortest period within which this organism has been known to divide, at least on artificial media, is about nineteen minutes. We have no observations to show that this phenomenon takes place in a very much shorter time in the animal body. Provided the organisms were just about to divide at the moment following their inoculation, it is questionable whether any of them would be alive to undergo the same process again at the end of nineteen minutes; but, as already stated, we do not know the exact period

at the end of which all of the organisms will have perished. Ordinarily, as we have seen, after an injection into the circulation of rabbits of a small amount of the cholera spirilla, the organisms after a few minutes can be obtained, if at all, only in slight numbers, from small quantities of the blood. This, however, does not necessarily mean that all of the spirilla in the animal body have been destroyed within this period. On the other hand, it would seem probable that the increase in virulence in the avirulent organism would begin at the moment of the inoculation. Theoretically, therefore, in the brief period of time preceding its destruction it would have an opportunity of increasing its haptophore groups and becoming more like the virulent strain. Still, whether this explanation outlined above is the correct one for this phenomenon can not be conclusively demonstrated without further experimental work and we must admit that we are at present unable, in an entirely satisfactory manner, to account for such a small variation in immunity after the employment of two strains of such different degrees of virulence. Perhaps additional light may be thrown upon the solution of this problem by the performance of similar experiments with other micro-organisms than the cholera spirillum.

It seems very evident that Profesor Welch's hypothesis is very applicable to the cholera organism in its relation to infection and immunity, and explains the reason why, as we have seen, it is only with great difficulty we are able to obtain even small amounts of intracellular toxine from our cultures on artificial media. It further explains how, in the animal body (particularly in the mucosa of the human intestine), the organisms, by the binding of suitable amboceptors to their own receptors, are capable of becoming much richer in endo-toxine and indeed of generating a considerable excess of it within a very brief period. Such a process applies also in the immunization of animals with the living organism, though from the observations made on the injection of the cholera spirilla into the blood circulation of animals it would seem that the bacteria do not find in the blood stream, etc., the same favorable stimulus for the production of the toxic receptors as they do in the cells of the mucosa of the human intestine. On the other hand, the proper amboceptors for the production of the bactericidal and agglutinative substances are here encountered. This conception also explains the difficulty which we have experienced (1) in obtaining large amounts of cholera antitoxine, and the relative ease with which

bactericidal substances are produced in the serum of the inoculated animals.

It would seem that the living avirulent strain by some process (not as yet satisfactorily explained in its entirety) is capable of increasing the number of its haptophore groups in the animal body after its injection into the circulation and before its total destruction; so that a relatively higher immunity is obtained by it than is produced when an organism of maximum virulence is employed. In other words, while in the case of the living organisms, a greater immunity is to be obtained from the more virulent strain, such immunity is not necessarily in direct proportion to the virulence of the bacteria used for the inoculation, as is the case, within certain limits, with the killed bacteria or with their free receptors.

CONCLUSIONS.

The virulent cholera spirillum possesses a greater number of bacteriolytic and agglutinable haptophore groups, or these groups are endowed with a greater binding power for uniceptors and amboceptors than the avirulent.

The number or the avidity of the bacteriolytic receptors possessed by a bacterium is directly proportional to its virulence.

However, the agglutinable receptors do not follow this law—that is, the agglutinable haptophore groups are not necessarily present in the same proportion as the bactericidal ones.

While the energy of growth is probably sometimes an important factor in relation to virulence, other phenomena must also be considered.

The virulent organism is possessed with a greater number of toxic haptophore groups than the avirulent.

The binding power of the free receptors of the organisms for bacteriolytic amboceptors in vitro is proportional to the bactericidal immunity produced in animals by each, which latter is in turn proportional to the virulence of the organisms from which the receptors were extracted. The binding power in vitro of the dead micro-organisms of different virulence for bacteriolytic amboceptors is not in proportion to their toxicity.

The bactericidal immunity obtained by means of the inoculation with the dead organisms of different virulence or their extracts (obtained by autolytic digestion) is proportional to the virulence of the living strains of the bacteria employed.

With the living organisms, while the bactericidal immunity obtained from the inoculation of animals with the virulent organism is greater than that produced with the avirulent, such immunity is not in direct proportion to the virulence of the bacteria introduced.

These conclusions apply to the two strains of cholera spirilla employed in the foregoing experiments. Whether they will also hold good with other strains of this spirillum or for micro-organisms in general, must be decided by further experimental work.

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ron.)	
1 12 000 14,000 15,000	Control Animals Without Serum.
A A D	6 control animals all neg: all dead in 24 hours.
AAAD	9 control animals all neg; all dead in 24 hours.
	5 control animals all neg; all dead in 24 hours.
	8 control animals all neg; all dead in 24 hours.
	7 control animals all neg; all dead in 24 hours.

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TABLE No. I

The average weight of rabbits, 1500 grams.

The injections were made into an ear vein with 1/2 oese of the living organisms suspended in 1 c. c. bouillon. All animals were killed by bleeding one week ofter inoculation. Agglutination experiments performed with both stems, virulent and avirulent, Bactericidal reactions performed only with the virulent stem.

Rabbit	Inoculated with	Agglutination Experiments.	Bactericidal reactions (Pfeiffer's Phenomenon.)	
No.	1/2 Oese intraven.	Ditulion of serum. Controls No. Conganism 1-50 1100 1200 1300 1400 1500 1500 1500 1700 1800 501., no serum	n . Dilution of serum	Control Animals Without Serum:
V.	*Virulent	wirulent W N N N N N N N N N N N N N N N N N N	AAAAAAAAAAAAAAAAA	6 control animals all neg: all dead in 21 hours.
VZ.	"Virulent"	"virulent" W W N N N N N N N N N N N N N N N N N	AA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	9 control animals all neg; all dead in 24 hours.
VII.	"Virulent"	Thed after five days. Agar plate cultures from all organs were storite		
VIII.	"Aviralent"	virulent WW N N N N N N N N N avirulent WW N N N N N	AAAAAAAAAAA	5 control animals all neg; all dead in 24 hours.
ZX.	"Aviralent"	viralent WW N N N N N N N N N N N N N N N N N N	A AAAAAA	8 control animals all neg; all dead in 24 hours.
X.	'Avirulent'	virulent W N N N N N N N N N N N N N N N N N N		7 control animals all neg; all dead in 24 hours.

7

= Complete agglutination. Overlying

Microscopically, positive bactericidal reaction.

Animal alive after 24 hours.

Microscopically, negative bactericidal reaction., Animal dead after 24 hours.

Bistinct agglutination with precipitation.

Overlying liquid not entirely clear.

Microscopically, Pfeiffer's Phenomenon doubtful; Animal alive after 24 hours.

non)	
118000 13000	Control Animals Without Scrum.
N/	2 control animals; reactions neg; all dead in 24 hours.
	1 control animal, reaction neg; dend in 24 hours.
	2 control animals; reactions neg; all dead in 24 hours.
	2 control animals; reactions neg: all dead in 24 hours.
9	2 control animals; reactions neg; all dead in 24 hours.
	1 control animal; reaction neg.; dead in 24 hours.

TABLE No. II.

Inocutations with tiving organisms average weight of rabbits 1150 grains.

(For explanation see Table No. I.)

Rubbu	Junculated with	Agglutination Experiments.		Bactericidal candions (Plaiffer's Phonomenon)	
No	1/2 Oese	Organism . Wilation of Serum.	Controls No Cl. Sol ; no secum	1 160 1 1000 13000 13000 1 1000 15000 16000 18000 18000 11000 11600 11600 11500 11500 11500 11500	Control Animals Without Serum.
194	Vivatent"	wratent X X X X X X X X X X X X X X X X X X X	N N N		2 control animals; reactions neg; all dead in 21 hours.
195		overalent W	N N	A A A A A A A A A A A A A A A A A A A	1 control animal, reaction ury; dedd in 24 hours.
196		virulent N N N avivalent WE N	N N N N	A A A A A A A A A A A A A A A A A A A	2 control animals; reactions neg; all dead in 24 hours.
197	Avirulent	virulent X X avirulent X X	N N N N	AAAAAAAAA	2 control animals; reactions neg: all dead in 24 hours.
1.98	Aviralent	vivalent N N avivalent W N	N N N N		2 control animals, reactions nog; all dead in 24 hours.
199	Averatent"	virulent X avirulent X	N N N N		1 control animal; reaction neg.; dead in 24 hours.

).	
10,000 12,000	Control Animals Without Serum.
	5 control animals; reactions neg.; all dead in 24 hours.
	4 control animals, reactions neg. all dead in 24 hours.
A	3 control animals; reactions neganil dead in 24 hours.
	4 control animals, reactions neg. all dead in 24 hours.
	3 control animals, reactions neg.; all dead in 24 hours.
	3 control animals; reactions neg; all dead in 24 hours.

TABLE No. III.

Inoculations with "Prophylactic" (For explanation see Table No.1.)

Rabbut	Inoculated with		lination Experiments.	MI .	Bactericidal reactions (Pfciffer's Phenomenon).	
No.	ICC. intraven.	Organism	Dilution of Serum.	Controls Na Cl sol; no serum	Dilution of Serum. 1.50, 1.00 1.200 1.300 1.400 1.500 1.600 1.700 1.800 1.900 1.000 1.2001 1.500 1.400 1.6000 1.5001 1.000 1.700	Control Animals Without Serum.
58	"Virutent" Prophylactic I	"cirulent"	N N N N	N N N N	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	5 control animals; reactions neg; all dead in 24 hours.
59	"Virulent" Prophylactic 1	"virulent" "avirulent"	W N N N N N W W W N N	N N N N		4 control animals, reactions neg. all dead in 24 hours.
60	Virulent" Prophylactic I	virulent" "wirulent"		N N N N	AAAA ABBE ABABI AAAAA	3 control animals; reactions negant dead in 24 hours.
61	Averatent" Prophylactic 1.	"mrulent" avirulent"	W N N N	N N N N		4 control animals, reactions neg. all dead in 24 hours.
62	"Avirulent" Prophylactic I."		N N N N N N	N N N N	AADADAAAAA	3 control animals; reactions neg.; att dead in 24 hours.
63	"Avirulent" Prophylactic I."		N N N N	N N N N	AVA VA VAVAVA A D	3 control animals; reactions neg; all dead in 24 hours.

enon.)	
000 23,000	Control Animals Without Serum
	2 control animals, reactions neg.; all dead in 24 hours.
	1 control animal; reaction neg; dead in 24 hours.
	2 control animals, reactions neg; all dead in 24 hours.
	1 control animal; reaction neg.; dead in 24 hours.

TABLE No. IV.

Inoculations with Prophylactic II.

(For explanation see Table No.1.)

Rathhy	Inoculated, with	$A_{\mathcal{G}}$	gglutination Experiments.		Bactericidal Reactions(Pfeiffer's Phenomenone)	
No.	12 C.C. intraven. (1. c C.= 1 0ese)	Organism	Dilution of Serum.	Controls Na.Cl. Sol; no serum	Dutution of Serum.	Control Animals Without Serum
86	Virulent" Prophylactic II. digested 2 das.	virulent' 'avirulent'	W N	N N N N		2 control animals, reactions neg; all dead in 24 hours.
87	Virulent Prophylactic II. digested 2 das.	virulent avirulent	W N N	N N	A A A A A A	1 control animal; reaction neg; dead in 24 hours.
88	Virulent Prophylactic II. digested 5 das.	virulent	W M	N N		2 control animals, reactions neg; all dead in 24 hours.
8.9	Virulent Prophylactic II. digested 5 das 8. reheated 2 hrs.60°C.	virulent	W N N	N N N N	A A A A A D D	1 control animal; reaction neg; dead in 24 hours.

nomenon)	
000 24,000 25,000	Control Animals Without Serum:
A JA D	6 control animals; reactions. neg; all.dead in 24 hours.
A	5 control animals; reactions neg: all dead in 24 hours.
	5 control animals; reactions. neg: all dead in 24 hours.
	4 control animals; reactions.

TABLE No. V.

Ineculations with Prophylactic III.

digested for five days.

(For explanation see Table No.1.)

Rabbil	Inoculated with	Agglutination Experiments.		Buttericidal Resettions (Soiffer's Phenomenan)	
No	GCC. intraven (1 GC. = 2 vese.)	Organism Dilution of Serum.	Controls No.Ct.	Dilution of Serum. 100 1 500 1000 2000 4000 5000 6000 10 000 15000 2000 2000 2000 25000	Control Animals Without Serum:
192	Virulent Prophylactic III.	ovirulent W W N	N N N N	Ala	6 control animals, reactions neg all dead in 24 hours.
193	Virulent Prophylactic III.	windent w N	N N		5 control animals reactions negral dead in 24 hours.
204	Avirulent Prophylactic III.	virulent W N N N N avivalent WE N N	N N N N		5 control animals reactions. nog. all dead in 24 hours.
205	Avirulent Prophylactic III.	vivulent W N N N avivulent W N N N	N N N N	A A A A V.	A control animals reactions. negrall dead in 24 hours.

rmenon)	
5,000 16,000	Control Animals Without Serum.
	Leastrol animal; reaction, neg., dead in 24 hours.
	1 control animal, reaction neg, dead in 24 hours,
	1 control animal; reaction negative, dead within twenty four hours.

TABLE No. VI.

Inoculations with Prophylactic IV.
digested for 2 days.
(For explanation see Table No. I.)

Rabbie	Inoculated	Agg	lutin	atio	n L	Ехере	erim	wnts					Bai	eleri	idal	Real	ction	s(Ple	uffer.	· Pho	mom	enon,	
No.	12 c.c. intraven-	Organismi	1-100 1		1.300			1.700	1-800	1-900		ls Na.Cl.		1000	12000	Dilut 0 1 400	ion es en	of S	0 12.00	00 130	00 15 00	0 16 000	Control Animals Without Sevan.
256	"Virulent"	virulent"						W	N	N	N	N							7:	1	1 1	1-1	I control animal; reaction
200	Prophylaetic IV.	"arirulent"					1		N	N	N	N		1	-		1/1	1/4	1	1	1/A	11	neg. dead in 24 hours.
257	Vivulent"	cirulent"	4	3				N	N	N	N	N			,	-			7 ±	A	4		I control mimal, reaction
201	Prophylactic IV.	"avivulent"		3				N	N	N	N	N		1			VA	A	14/1	10	i		neg dead in 24 hours,
177	Aviralent'	oirnlent"			N	N	:		1	;	N	N				N. N	1		1	-	;	1	
211	Prophylactic IV.	"avirulent"	5	Ž		N	-	1	1	!	N	N	Va.	VA	VA	1/0	1	1	1		:	:	1 control animal, reaction
178	"Avirulent"	"virulent"		w	N	N	1	1	-		, N	N	19		N. N	1:	i	1	i		-		negative, dead within twenty four hours.
	Prophylactic IV.	'avirulent'	Telimenti XI		W	N			!		N	N	A	A	1/0	1	-	:	-	:		-	taking jour neurs.

renon.)

9.000 10.000 12.000	Control Animals Without Serum.
	1 control animal; reaction neg.; dead in 24 hours.
	1 control animal; reaction ney; dead in 24 hours.
A D	1 control animal; reaction neg.; deed in 24 hours
N/D	1 control animal; reaction neg; dead in 24 hours
	1 control animal:reaction negs dead in 24 hours.
	I control animal; reaction neg dead in 24 hours

TABLE No. VII.

Subcutaneous Inoculations with Prophylactic (For explanation see Table No. I.)

Pahbut	Inoculated	Agglutination Experiments.	i	Bactericidal Reactions (Yeiffer's Phenomenon)	
No.	Subsulaneously with.	Organism Pullition of Serum.	Controls Na.Cl	Dilution of Serum. 1 100 200 1 300 400 500 700 900 000 400 500 600 7000 8000 9000 1000 12000	Control Animals Without Serum.
399	See Veralent Prophylactic V	virulent W W N svirulent N	N N N N		1 control onimal reaction negs dead in 24 hours.
100	500. Vivulent Prophylactic V.	virulent W W N N avrulent W N N N	N N		1 control animal; reaction neg; doad in 24 hours.
123	Sec Virulent Prophy lactic V. Preserved in 5% carbolic acid	oirulent WWW N	N N N N		1 control animal; reaction neg; dead in 24 hours
426	200 Virulent Prophy. tactic V. reheated tagget, for 30 min.	virulent N N avirulent N N	N N	A VA	1 control animal reaction neg; dead in 24 hours
174	Sec. Avirulent	virulent N N avirulent N N	N N		1 control animal reaction neg dead in 24 hours.
184	Sec. Avirulent Prophylactic V.	virulent W N	N N N N		Leasted animal; reaction neg: dead in 24 hours
440	Sec Virulent Prophy. lactic VI. in Chloro. tone (150 = 8 oese)	nivulent W N N avirulent	N N	A A JA JAYAYAY	

non)	
4000 1-5000	Control Animals Without Serum
A /n	2 control animals; reactions neg. all dead in 24 hours.
	2 control animals; reactions neg.; all dead in 24 hours.
	2 control animals; reactions neg: all dead in 24 hours.
	2 sontrol animals; reactions neg; all dead in 24 hours.
	1 control animal; reaction neg.; Jead in 24 hours,
	I control animal; reaction neg, dead in 24 hours.

TABLE No. VIII.

Inoculations with Dried Prophylactic redisolved in Na Ct sot. (For explanation see Table No. I.)

Rabbu	Invoutated	Agglutination Experiments.		Bacterioidal Reactions (Pfeiffer's Phenomenon)	
No	Dried Prophylac,	Organism Vilution of Serum.	Controls Na.Ct.	Dilution of Serum. 150 1100 1200 1300 1400 1500 1600 1700 1800 11000 17000 13000 14000 18000	Control Animals Without Serum
167	10 Mgs. Virulent II.	oiruled Y ovirulent Y	N N N N		2 control animals; reactions neg: all dead in 24 hours.
168	3 Mgs. Virulent II.	virulent / N N	N N N N		2 control animals, reactions negacit dead in 24 hours.
187	(Subcutaneous) 5 Mgs. Virulent III.	circulent N N accordent W N	N N		2 control animals; reactions neg: all dead in 24 hours.
16.9	10 Mas. Neiralent II.	vicutent N N N oviralent W N	N N		2 control animals; reactions neg; all dead in 24 hours.
170	3 Mys Avivulent II.	ovirulent N	N N N N N N N N N N N N N N N N N N N		1 control animal: reaction neg.: dead in 24 hours.
175	(Subcutaneous) SMys Avirulent III.	virulent W N N	N N N N	AVAVAVA	1 control animal; reaction Roy, dead in 24 hours.

Control Animals Without Serum

trol animal, reaction, dead within 24 hrs.

trol animal; reaction

rol animal; reaction; dead within 24 hrs.

rol animal; reaction; dead within 24 hrs.

TABLE No. IX.

Showing Bactericidal value of Guinea pig serum after treatment for 2 hrs. with the virulent and avirulent strain.

Original Bactericidal value of Serum.... 1-9,000 Fost Mice 1-10,000 Neg. Dead

L	Original	St.c. of Serum.		Bactericidal Reactions (Pfeiffer's Phenomenon.)															
	Dilution of Serum.	Centrifuged with	1.100 1.20	0 1-300	1-4,00					n of			6,500	7,000	8.000	8.500	9,000	10,000	Control Animals Without Serum
	1-20	5 Oese Virulent	AAA	A	A	A	N/D	N D			:								1 control animal, reaction neg; dead within 24 hrs.
	1-20	5 Oese Avirulent	A			1			A	A	A	A	A	A	A	A	N/D	N/D	1 control animal; reaction neg; dead within 24 hrs.
	1-100	5 Oese Virulent	A		A	A	A	N/D											1 control animal; reaction neg; dead within 24 hrs.
	1-100	5 Oese Avirulent	A			A		-	A		V _A			A	A	A	N/D		1 control animal; reaction neg.; dead within 24 hrs.

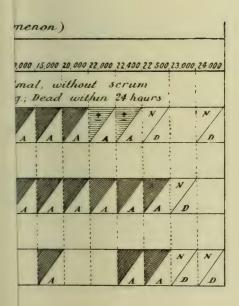


TABLE No X.

Showing Bactericidal value of Rabbits serum after treatment for 2 hrs. with the virulent and avirulent strain Original Bactericidal value of serum.... 1-24.000 Post. Nive 1-25.000 Neg. Dead

Original	Sc & Serum	Bactericidal Reactions (Pfeiffer's Phenomenon)
Dilution of Serum	Centrifuged with	Actual dilution of Serum 1-80 1160 1-300 1-700 1-800 1-900 1,000 1100 1200 1400 2400 2500 2800 4000 5000 6000 8,000 10,000 15,000 22,000 12,400 22,500 23,000,24,000
1.20	5 Oese Vicutent	1 Control animal, without scrum reaction neg; Dead within 24 haurs
1-20	5 Oese Avirulent	1 Nontrol animal Dead within 24 hrs
1-100	5 Oese Virulent	1 Control animal Dead within 24 hours.
1-100	5 Oese Avirulent	1 Control unimal. dead within 24 hours.
1-500	5 Oese Virulent	1 Control animal dead within 24 hours.
1-500	5 Oese Avirulent	1 tontrol animal dead within 24 hours.

nt

4,000	Control Animals Without Serum.
	1 Control; reaction neg; dead within 24 hours.
N/D	1 Control; reaction neg.; dead within 24 hours.

TABLE No.XI.

Showing Bactericidal value of Rabbit serum after treatment for 2 hrs. with the Killed virulent & avirulent organisms.

Original Bactericidal value of serum.... 1-24,000 Post. Alive 1-25,000 Neg. Dead.

Original	5 c.c. Serum.	Bactericidal Reactions (Pfeiffer's Phenomenon.)	
Dilution of se- rum + bouillon.	Centrifuged with	Actual dilution of serum. 1-200 1-400 1-400 1-1000 2.000 3.000 4.000 5.000 6.000 1,000 15000 20000 21,000 22,000 24,000	Control Animals Without Serum.
1-40	5 Oese Killed Virulent in bouillon		1 Control; reaction neg; dead within 24 hours.
1-40	5 Oesc Killed Avirulent in bouillon		1 Control; reaction neg.; dead within 24 hours.

nt

Control Animals
Without Serum.

1 Control; reaction neg.; dead within 24 hours.

1 Control; reaction neg; dead within 24 hours.

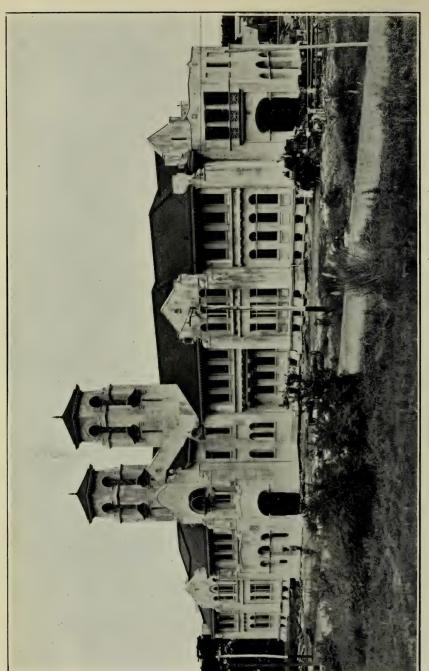
TABLE No. XII.

Showing Bactericidal value of Rabbit serum after treatment for 2 hrs. with virulent & avirulent Prophylactic IV.

Original Bactericidal value of serum. [1-24,000 Post. Alive 1-25,000 Neg. Dead

Original Sc.c. of Serum. Bactericidal Reactions (Pfeiffer's Phenomenon.)													
Dilution of serum + Prophylactic	Centrifuged with	1-1,00	1-1,000	1-5,000		al dil 1-10,000		-			23,000	24,000	Control Animals Without Serum.
1-40	5 c.c." Virulent" Prophylactic IV.	A	A	A			A	A	N D				1 Control; reaction neg.; dead within 24 hours.
1-40	5 C.C. Avirulent Prophylactic IV.			A	A					A	1	N/D	1 Control; reaction neg.; dead within 24 hours.





VIEW OF THE FRONT ELEVATION OF THE BUILDING. (FRONTISPIECE.)

No. 22.-1905

DEPARTMENT OF THE INTERIOR BUREAU OF GOVERNMENT LABORATORIES

I. DESCRIPTION OF NEW BUILDINGS

BY PAUL C. FREER, M. D., PH. D.

II. A CATALOGUE OF THE LIBRARY

BY MARY POLK, LIBRARIAN

MANILA BUREAU OF PUBLIC PRINTING 1905



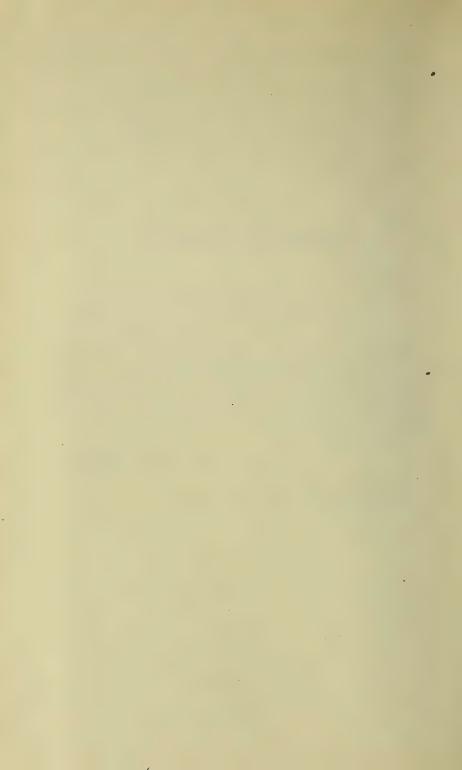
LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, October 20, 1904.

SIR: I have the honor to transmit herewith a paper entitled "I. Description of the New Buildings of the Bureau of Government Laboratories," by Paul C. Freer, M. D., Ph. D., Superintendent of Government Laboratories, and "II. A Catalogue of the Library of the Bureau of Government Laboratories," by Mary Polk, librarian. I am, very respectfully,

Paul C. Freer,
Superintendent of Government Laboratories.

Hon. DEAN C. WORCESTER, Secretary of the Interior, Manila, P. I.



I. DESCRIPTION OF THE NEW BUILDINGS OF THE BUREAU OF GOVERNMENT LABORATORIES.



PART I.

THE ORGANIZATION AND THE BUILDINGS OF THE BUREAU OF GOVERNMENT LABORATORIES.

INTRODUCTION.

Tropical countries which are subject to colonization by the white races present conditions which are such that the settlers are continually exposed to infectious diseases, differing from those prevalent in colder regions, and, owing to the fact that the European races in these countries have been moved from their native soil they are exposed to greater dangers than the native population, which have become accustomed to the surroundings. However, it is true that the natives contract the same class of diseases and are subject, though perhaps in a lesser degree, to the same dangers. localities are especially prone to the occurrence of serious epidemics both of man and of animals. In all such countries the native population regard the causes of the sicknesses which afflict them from a standpoint entirely different from that of the educated white man. Superstition and imperfect education leads them to disbelieve in measures which the modern scientific world has recognized as necessary. For this reason, health boards, modeled more or less after those of the mother countries but modified as to their powers and duties in order to meet existing conditions, have been deemed essential in all tropical colonies, but their work to a very great extent would be imperfect, haphazard, and unsuccessful if the methods of prophylaxis were not guided and sustained by a scientific knowledge of the causation of disease and by accurate diagnoses of the exact nature of the infections which are encountered. From a material standpoint, the damage to commerce caused by epidemics of cholera, plague, rinderpest, and surra is such that the loss where these diseases gain a foothold by far outweighs the cost

of any measures of prevention, however expensive. For the purpose of diagnosis and for the further study of methods of prophylaxis and cure, a biological laboratory, properly equipped, is an The work is of so difficult a nature, so important, and, if imperfect methods are used, so subject to error, that a poor equipment both in the literature of medical biology and in apparatus would be the precursor of failure. The appliances must be of the best, the literature on all branches of the work must be at hand, and the bacteriologists performing the diagnoses must have a thorough and complete training. However, bacteriological laboratories in the Tropics must not confine themselves to subjects of diagnosis—they must also be expected to make advances by research on special subjects of importance, and they must continually enlarge their field by the observation of interesting or new diseases which may come to their attention, because it can never be predicted that an apparently harmless malady which is imperfectly known may not eventually assume serious proportions. In so doing the laboratories can guard the public by timely warnings. Each tropical colony presents different conditions, different phases of the same disease, and even new and unknown infections. The prevention of the spread of any disease must presuppose a perfect knowledge of its etiology and of the other factors which enter into its prevention and treatment, and such knowledge can only be gained by an investigation of the subject based upon a study of the literature and supplemented by new investigations in the laboratory. Research in the field outlined above demands the highest type of trained investigators, a complete library, and exceptional facilities. The value of this class of work is so universally recognized that governments in the past have organized expensive expeditions to tropical countries for the purpose of increasing the world's knowledge of the diseases peculiar to those regions. However, such expeditions can not possibly carry with them all the materials for their work without encountering great difficulties which rob them both of time and opportunity, and their results would be far more beneficial if they could come to permanent stations.

For a properly equipped biological laboratory there are necessary not only adequate rooms, well lighted without direct sunlight, but also apparatus such as microscopes, incubators, sterilizers, microtomes, surgical instruments, glassware of all kinds, stains, chemicals, and small animals. The latter, such as guinea pigs,

rabbits, monkeys, goats, and dogs, are as much a portion of the laboratory reagents as are the culture media in which the bacteria grow or the microscopes through which they are seen.

Tropical colonies are also of necessity regions the prosperity of which depends upon agriculture, upon an exploitation of the natural products, upon mining interests, and the means for the barter and sale of raw products. They can not under present conditions, or presumably even in the remote future, become manufacturing centers excepting for products closely related to their chief staples. It is a fact that the interior of many of these countries is but little known, that the products are gathered by persons ignorant of their true uses and values, that in many cases they are allowed to go to waste for want of a proper knowledge, and that in others the exploitation is desultory and imperfect. Some phases of tropical agriculture are fairly well advanced; among these may be mentioned the culture of the cocoanut, of hemp, rice, etc., but when it comes to articles of a different character, which are obtained from the forests or mines, it is found that much uncertainty exists. Gums and resins, which are supposed to be of but little value to the natives and which they may use for fuel or for lighting purposes, may, on distillation, produce substances of far higher commercial value. Medicinal plants may exist in large numbers with qualities unknown or imperfectly known to the people, but containing alkaloids and other materials which if properly investigated would be readily marketable at high prices. fields of enterprise, based upon knowledge secured in other countries, may also be introduced. In order to gain advantages not heretofore obtained in the conservation and exploitation of natural products or of those introduced for the purposes of culture, bureaus of forestry, agriculture, and mines and commercial museums have been established, but all of these need the assistance, advice, and research work of laboratories to answer questions as to the nature of soils and minerals, the value of ores, the uses and composition of oils, gums, resins, and medicinal plants, and without such assistance the value of their work would be but a fraction of what it is.

For the purposes of study of the subjects mentioned above a necessity exists for a laboratory of chemistry and for facilities to pursue and complete accurate researches on the materials which can be obtained. In the Tropics, as well as in other countries, the

question of the character of the foods and drinks, the analysis of imported products which too frequently are adulterated, the determination of values for custom duties, the testing of the strength of cements, the assaying of ores, and the performance of other analytical work are necessary, and, as these regions are not supplied with universities, analytical laboratories must be provided in charge of the various governments.

As the people depend upon products of the field and of the forests for so large a proportion of their sustenance and barter, a knowledge of the flora of the Tropics is essential, from both a scientific and a material standpoint. It is necessary to be able to identify plants which have once been encountered in order to understand something of their distribution and general importance, to study their growth, the conditions necessary for their best development, and their diseases. Bureaus of forestry and agriculture must depend upon botanical work to assist them in their duties. The chemist, in investigating plant products, needs an identification of the plants from which the latter come. For these reasons botanical work becomes as essential as that in any other laboratory field.

Tropical countries are, par excellence, those in which insect pests are most frequent and widely distributed, so that the means for the study of entomology, facilities for classifying the material gathered, and for ascertaining the nature of the insects most dangerous to valuable flora should be at hand, and this need must be met by the employment of entomologists and by providing space for their work.

Modern methods for the treatment of disease have become more and more dependent upon serum therapy. The results obtained with rinderpest in South Africa and in India, with plague prophylactic in Japan, with cholera prophylactic in India and Japan, and the necessity for vaccination against smallpox, at once suggest the establishment of serum laboratories in tropical countries. Because serums are perishable they can not very successfully be shipped through long distances, and many of the tropical diseases which yield either to serum prophylaxis or therapy are of such a nature that the serums themselves can not be prepared except on the spot.

Other branches of laboratory work readily suggest themselves as being equally essential to the proper development of colonial enterprise. One of these would be a study of the fauna of the regions in question, both marine and terrestrial. A laboratory for zoölogy

and marine biology would be entitled to equal rank with the others mentioned above.

A government in beginning its work can adopt one of two lines of action. It may either allow its various divisions which come in contact with and which need scientific aid themselves to establish the various laboratories in question, each under separate direction and each with separate facilities, or it may adopt the course of inaugurating one central institution where all this class of work can be united, where, therefore, the workers can be in close contact, and where each division is well aware of what is being done in the others. Coöperation in this sense would be most complete, and while there might be some slight disadvantages in the latter course by reason of the fact that the various divisions of the government could not in their own quarters study and obtain the results looked for, these are by far outweighed by the cooperation which can be obtained between the scientific men by the division of labor, which frequently saves both time and money, and by the reduction in equipment, which inevitably follows a concentration of allied interests.

The Civil Government of the Philippine Islands, taking all of the above-mentioned facts into consideration, and knowing well the expense and loss of efficiency due to a scattering of its scientific energies through a number of bureaus, decided to establish one central laboratory system, to properly equip and house this series of institutions, and to place them under a central direction.

By Act No. 156 of the Philippine Commission, passed in July, 1901, there was established a Bureau of Government Laboratories, consisting of a biological laboratory and a chemical laboratory. Later, by Act No. 607, there was united therewith a serum laboratory. Subsequently the botanist of the Agricultural Bureau was transferred to the Bureau of Government Laboratories and the botanical staff was enlarged, so that another division was added to the biological laboratory. Entomological investigation was also begun, and of late zoölogical work has been undertaken.

By Act No. 156 the Superintendent of Government Laboratories was directed to prepare plans for a suitable building for the installation of the biological laboratory, the chemical laboratory, and a reference library; for a laboratory for the manufacture of vaccine virus, serums, and prophylactics; detailed estimates of the cost of

the buildings and of properly equipping the different laboratories, and of procuring a reference library. Pursuant to these directions the Superintendent of Government Laboratories, soon after his arrival in the Philippine Islands in September, 1901, with the assistance of the Chief of the Insular Bureau of Architecture, Mr. E. K. Bourne, and the Director of the Biological Laboratory, began the preparation of the plans.

Discussion of the proper type of building to be used for laboratory purposes of various kinds, the question as to whether large general rooms or small individual ones are more advantageous, the consideration of the necessities for modern laboratory work, and the other general phases of the struggle for perfection in laboratory construction has of late occupied a considerable field in the deliberations of scientific bodies, and it may be said that there are as many views as to what is necessary and as to what means should be employed to reach given ends in laboratory work as there are laboratory workers. The day has gone by when scientific research can be successfully conducted on the kitchen stove. Those things which could be done with simple appliances and with simple or imperfect equipment have been done. Modern scientific theories and the facts upon which they are based are the product of investigation which uses every possible means to obtain the end sought, and are based upon exact and accurate measurements. To accomplish such ends at the present time requires an equipment far beyond that ever dreamed of, or possible, fifty years ago, and it is because of this rapid development that the discussion of laboratory construction has become an important one. As the new Government Laboratories in Manila were to signalize a departure in the policy of tropical governments, by uniting all laboratory work in one bureau, and, as the buildings of necessity were designed to meet many ends, it has been deemed not superfluous to describe the structures as finally erected and to bring before the scientific world such phases as may be new and interesting.

The question as to whether the laboratory buildings should contain large general rooms or should be composed of smaller apartments for scientific work was decided in favor of the latter method of construction, for the following reasons: The Bureau is not expected to handle large classes of students and consequently lecture rooms could be omitted; and the variety of its work is such

that in any event a considerable separation of the men would be necessary. At most no more than four workers in the same line would presumably need space at the same time, and as all laboratories would be in one building, the freedom of contact and opportunity for discussion would not materially be curtailed, although each class of work could be completely separated from all others. These arguments were so strongly in favor of smaller and numerous rooms that the main structure was planned accordingly. The decision to place all the laboratories in one building was made in order to secure compactness, ease of administration, and to foster in the highest degree the personal contact of the scientific workers in the laboratory. While there may be some argument in favor of separating biological and chemical laboratories, nevertheless, by properly planning the building the work can be divided in different wings, and by supplying adequate hoods and drafts it is certain that all classes of work can be placed under one roof without the one interfering with the other.

As the building was to be in the Tropics, it was necessary to pay great attention to ventilation and coolness, so that in the main structure the rooms were grouped on either side of a large main corridor 10 feet wide and running the entire length of the building. As this hallway is open at either end, a breeze is almost continually passing through it, generally supplying a suction as it passes the doors of the individual laboratories so that a constant circulation of air is produced. The building was designed to be of no more than two stories, both because of the fact that the Philippine Islands are subject to earthquakes and also because previous experience has demonstrated that unless great care is taken the laboratories on the top floor, if leakage or breakage of containers of liquids should occur, are apt to interfere with the work below. This danger is minimized in a two-story structure.

When the work of preparing the plans was first undertaken it was decided to be expedient and necessary to install a power plant of a capacity to provide all of the rooms with vacuum, air pressure, and steam, and as up to the present time the alternating current furnished by the Manila electric-light works has been unsatisfactory for laboratory purposes, it was also planned to give sufficient power to light all the laboratory buildings. While the discussion was being carried on in regard to the amount of power necessary,

it was decided to add two wings to the building in the future, one to accommodate the Bureaus of Forestry and Public Lands, the other those of Agriculture and Mining, so that an enlargement of the power plant in order to meet the increased demand was called for. Finally, in considering a site for a hospital in Manila, it was decided that the Bureau of Government Laboratories, by its very nature, should be located in proximity to the institution which it was proposed to establish, not only because laboratory work is an essential to success in modern hospitals, but also because of the economy which would be brought about by having a central light and power plant for all of the structures which were to be grouped together. This plan necessitated a further increase in the machinery and equipment.

The location of the building was for a long time uncertain. The intention at first was to place the laboratories on the high land at Santa Mesa to the north and east of the city of Manila, a situation which was ideal in every respect excepting that of distance. Santa Mesa there were abundant lands, but unfortunately the title thereof was involved, and for this reason, unless the Government wished to wait a long time for its adjustment, the site was unavailable. In consequence, the old Exposition Grounds, between Calle Herran and Calle Padre Faura, of an area of approximately 24 acres, were decided upon. This land is much nearer the heart of the city than is the other location; it is level, although somewhat low, and its situation is such that were a hospital to be placed on it the length of the trips for the ambulances and for the patients would be minimized. The tract of land is also of sufficient size to accommodate not only the laboratory buildings and all future wings but also a proposed medical college and the necessary hospital structures. The location having been decided upon and a small additional tract of land, which was necessary in order to round out the Exposition Grounds into a regular piece, having been purchased, it became possible to begin the structure at once. Previous to this time the plans had been carefully prepared, including not only those of the building and power house but also those of the necessary laboratory desks, hoods, and appliances of all kinds. They had been presented to the Philippine Commission, approved by them and the Civil Governor, and work on the foundation was begun in October, 1902.

PLAN OF THE BUILDINGS.

The buildings were divided into a main laboratory structure, facing toward the south and divided into two symmetrical portions, the one on the east for the biological and the one on the west for the chemical laboratory. The power house was placed to the rear and connected with the main structure by means of a corridor, and in addition to the space for the boilers and engines it provides room for the serum laboratory.²

The whole structure, therefore, is in the form of the letter T, that portion of it in which biological and chemical work is carried on being so planned as to give the minimum of direct sunlight—a great desideratum for microscopic work. Bisecting the main structure lengthwise are three corridors 10 feet wide and 236 feet long, the one in the foundation being for the purpose of giving access to all the pipes leading from the power house and to provide space for the storage battery. The two upper hallways are the main passageways of the building and all of the rooms open on them. The eastern half of the structure is devoted to biological work with the exception of the space occupied by one room (No. 10) for storage of apparatus and another for offices (No. 13). Passing through the main entrance and turning to the right, the disposition of the rooms is as follows: No. 12 for the preparation of culture media for biological work, No. 14 for botanical work, No. 18 as a storehouse for botanical duplicates, No. 19 a mechanic's room, Nos. 17 and 15 for bacteriologic diagnosis and research. On the upper floor in the same order No. 116 is for the photographer, No. 118 for the pathological museum, No. 120 for pathological work and research, No. 122 for incubators and refrigerators, No. 124 for entomological work, No. 121 an outdoor laboratory for the entomologists, No. 119 a laboratory room for bacteriological and pathological research, No. 117 for biological research, and No. 115 as an office for the Director of the Biological Laboratory. Turning to the left from the entranceway on the ground floor, room No. 8 is for the storage of chemicals and apparatus; No. 6 as a laboratory for organic chemistry in connection with drugs, medicinal plants, and natural products; No. 4 for a commercial laboratory in chem-

¹ See Pls. I and II.

² See Pl. IX.

istry, with the machinery for carrying on commercial processes on a laboratory scale; No. 1 for photometric work; No. 3 for physical chemistry and physics; No. 5 for the same purposes and for the adjustment of weights and measures; No. 7 for assaying and mineral work; No. 9 for a balance room, and No. 11 for organic combustion. On the second floor and in the same order No. 114 is the balance room for the upper floor; No. 112 for the use of the ornithologist; No. 110 is a chemical laboratory fitted for work with soils, water, and organic chemistry; No. 108 a chemical laboratory fitted for all classes of work; No. 106 for spectroscopes and instruments of precision; No. 104 for sugar, foods, and physiological chemistry; No. 103 for mineral analysis; No. 105 for general chemical work of all classes, and No. 107 as an office for the Superintendent of Government Laboratories. The three central rooms to the front-Nos. 109, 111, and 113-are for the purposes of the library, Nos. 109 and 113 being stack rooms and No. 111 the reading room, stack room, librarian's office, and general library. Passing to the rear on the ground floor and turning to the left in the power house, Nos. 50 and 51 are for cold storage, No. 52 for the packing and shipping of vaccine and serums, No. 53 for the preparation of serums, No. 54 as a room for the preparation of culture media for the serum laboratory, No. 55 as a boiler room, No. 56 for the gas-plant generators, No. 57 for coal, No. 58 for a crematory, No. 59 for the engine room, and No. 60 for coldstorage machinery. No. 153 is the office of the Director of the Serum Laboratory and Nos. 150 and 152 are laboratories for bacteriological and pathological work of the serum laboratory. To the rear of the entire structure are placed the animal houses, including buildings for the storing of guinea pigs and rabbits, with a wing for work on bubonic plague and contagious diseases; a stable for the serum horses, one for the vaccine calves, and one for the storing of monkeys, dogs, and goats.1 The list given above completes a statement of the purposes for which the rooms were originally intended, but the detailed description which will be given below makes it evident that the building is really so constructed as to give it great elasticity in the class of work which can be conducted therein and in providing for a large increase in the number of workers.

¹ See Pls. III-VI.

POWER EQUIPMENT.

The boiler-room equipment consists of two 75-horsepower Babcock & Wilcox steel sectional boilers set in one battery and designed for a working pressure of 150 pounds per square inch. Steam is led from the boilers through 4-inch copper-expansion bends to an 8-inch extra-heavy steel-pipe header, from which a 7-inch steam main drops to the pipe subway. The engine supplies are 3-inch copper pipes tapped into the 7-inch main and carried into the engine room below the floor line, from where they are carried up to the cylinders by expansion bends of 4-foot 4-inch radius. The exhaust main is 8-inch standard pipe, and passes through a 200-horsepower Wainwright even-flow feed-water heater, which is placed in the subway, and from the latter it passes through the boiler room to the atmosphere, being fitted with a 24 by 30 inch bent exhaust head. The main steam and exhaust lines are drained by two Bundy returnsteam traps discharging into a feed-suction tank located in the subway.

The flue gases from the boiler are led through a sheet-steel smoke header to the main smokestack, which is of 48-inch internal diameter and 103 feet high. A damper is fitted into the smoke header which is automatically controlled by a Locke hydraulic damper regulator. A 24 by 60 inch vertical auxiliary boiler, with a Deane 31 by 4 inch duplex steam pump, is installed for general water service during periods when the main boilers are shut down. The feed water for the main boilers passes through a Loomis-Manning Type H pressure filter, which discharges into a filter tank having a capacity of 500 gallons, from which it is carried to two 111-inch Metropolitan double-tube injectors fitted to the main boilers, the auxiliary boiler and pump, and to the main feed pump, which is of the Deane triplex single-acting type, with cylinders 4 by 4 inches geared to a Stow multispeed motor. The pump and motor are located in the pipe subway, and receive water from the steam traps and cylinder jackets of the air compressor, vacuum pump, and ice machine, as well as from the filter tanks.

The fire pump is of the Deane duplex inside-plunger pattern, 7½ by 4½ by 10 inches, with 4-inch suction and 3-inch discharge to the fire-main system in the main building. A connection is also made from this pump to the house-service tanks, two in number,

having a combined capacity of 900 gallons, located on the south side of the boiler room. The tanks are so connected that they may be used either together or singly. Coal-storage capacity of 30 tons is provided on the north side of the boiler room.

Two Mansfield oil gas generators of a capacity of 100 cubic feet per hour each are installed close to the coal bunkers and deliver gas to two sheet-steel gas holders, each of 1,250 cubic feet capacity, located 100 feet from the power-house building. A 12-inch smoke header connects the gas-generator furnaces to the main stack.

A cremating furnace 10 by 5 by 6 feet is built at the foot of the main stack, having 12 square feet of grate surface and an incinerating chamber of 4 by $2\frac{1}{2}$ by $2\frac{1}{2}$ feet for burning laboratory refuse, small animals, etc.

All piping, conduits, etc., connecting the various apparatus in the boiler room pass through a concrete trench covered by checkered-steel floor plates to the pipe subway, from which point they are carried to the basement corridor in the main building. The piping ranges in size from one-fourth inch to 8 inches, and wire conduits from one-half inch to $2\frac{1}{2}$ inches. The subway and corridor are automatically drained by Lawlor cellar ejectors worked by water pressure.

The west end of the main basement corridor contains a storage battery of 20 cells from which current is conveyed to various laboratory rooms.¹

The engine-room equipment consists of two horizontal single-expansion Ideal automatic engines of 60 horsepower each, direct connected to two Westinghouse $37\frac{1}{2}$ -kilowatt six-pole compound-wound direct-current generators. Each unit has a capacity of 300 amperes at an electromotive force of 125 volts at a speed range of 290 to 320 revolutions per minute. The generators are fitted with equalized connections for parallel working. Current is led from the generators through loricated-steel conduits laid below the flooring to a 5-panel black slate switchboard 15 feet long. Each generator is protected by an I-T-E laminated contact circuit breaker. The switchboard has two-generator one-power distributing and one light-distributing panel, and one panel for instruments; it also carries all the air, gas, vacuum, water, steam, and electrical indicating and recording gauges. Ten light-and-power feeders are

¹ Pls. X and XI are photographs of the boiler rooms.

carried from the distributing panels by means of 2-inch loricatedsteel conduits through the subways to eight distributing panels located in the corridors of the main building, and two power feeders pass through conduit to the towers to supply the exhaust-ventilator motors.

Compressed air is supplied by a No. 21 "Christensen" Type N 8½ by 8 inch horizontal compressor of 75 cubic feet per minute capacity. The compressor is direct geared to a 14-horsepower series-wound four-pole motor, and is automatically controlled between any predetermined maximum and minimum pressure. The air receiver is 3 feet in diameter by 10 feet high and is located in the boiler room.

The vacuum pump is a 7 by 7 inch "Clayton" type, and is geared to a General Electric CE type direct-current 3-horsepower motor and will maintain a vacuum in the pipe system of the main building of approximately 29 inches.

The house-service pump is a No. 4 "Quimby" type, direct connected to a General Electric CE type 15-horsepower motor, automatically controlled between any predetermined maximum and minimum pressures up to 125 pounds per square inch. The pump has a 4-inch suction and a 3-inch discharge to two 36 by 120 inch compression tanks located in the boiler room, from which the water is distributed to the main building and animal houses.

The refrigerating machinery consists of a "Brunswick" double-cylinder single-acting ammonia compressor of 3 tons refrigerating capacity, direct connected to a General Electric eight-pole slow-speed motor, a double-pipe ammonia condenser, oil separator, ammonia receiver, with combination gauges, thermometers, etc., located in the south end of the engine room. The brine tank is 4 by 6 by 14 feet and contains five 1-inch ammonia expansion coils; the chilled brine is circulated by a "Quimby" No. $2\frac{1}{2}$ rotary brine pump, direct connected to a General Electric CE type 1-horsepower motor, and passes to two cold-storage rooms having a joint capacity of 3,000 cubic feet and to two refrigerating boxes in the main building each having a capacity of 200 cubic feet. Each of the main storage rooms has five refrigerating coils of $1\frac{1}{4}$ -inch galvanized pipe and will be maintained at a temperature of 28° F.

Two 15-inch "Buffalo" B volume No. 6 exhaust fans, direct connected to General Electric CE type 5-horsepower motors, are located in the towers of the main building and connect with a

16-inch exhaust main in the attic, from which 4 and 6 inch branches are carried down to the hoods on the first and second floors. The motors are operated from the main switchboard by means of solenoil-controlled switches and self-starting rheostats.

The exhausters will maintain a velocity of 1,800 feet per minute through the mains and will allow a complete change of air every minute in all the hoods.¹

Starting from the power house all the main distribution of the building is through a subway which is in the form of the letter T. the portion entering from the power house joining that from the main building at about the middle. The diameters of the piping leading to the various laboratory rooms have been calculated so as to give as equal a supply as possible of gas, water, vacuum, air pressure, and steam in the various rooms. In order to effect this, the water and gas mains form loops on each floor of the two wings of the main building, thus supplying a perfectly even circulation, and the taps to the individual rooms and desks are taken from these loops. The electric-light wires as well as those of the storage battery and telephones are carried throughout in steel conduit and distributed wherever needed, so that each desk is supplied with light and electric power, provided the motors to be used do not exceed one-sixteenth horsepower. Where greater power than this is necessary, separate lines have been run into the building. The storage-battery current is supplied only to those rooms where presumably it will be necessary; for example, those for mineral analysis, physics, and weights and measures, the spectrum analysis room, and the private laboratory of the Superintendent of Government Laboratories.

THE DESK ARRANGEMENTS IN THE INDIVIDUAL ROOMS.

The general plan and assignment of the rooms has been mentioned above, so that only a description of the individual arrangements is necessary. As these vary but little throughout the biological and chemical wings, a description of one room for each section will, with few exceptions, do for the others. In the biological wing a microscope table 32 inches above the floor and 30 inches wide is provided along the entire available window front.

¹ Pl. XII is a photograph of the engine rooms.

Each one of these contains two small sinks let in at convenient points, and is furnished with water and gas. Those portions of the tables directly facing the windows are not supplied with drawers, the latter being provided in those sections which presumably, by reason of their not being directly centered behind windows, would not be used for microscopic work. The windows have been placed approximately 16 inches above the level of the desks, so that the strong breezes which prevail in this country would not play havoc with the materials on the work table, but at the same time the light is ample. In the center of each room devoted to biology is a large double work desk supplied with gas, water, and vacuum, with a large sink and drip board at one end. These desks are 36 inches high and are intended for the general work of the laboratory, providing facilities for heating, filtering, distilling, etc. Each one of these central tables has closets underneath and drawers. To one side, along the wall of each biological room, is a chemical work table furnished with gas, water, and vacuum connected with a sink and having a trough extending the length of the desk; the opposite wall is occupied by a hood 8 feet in length, equipped like the side desk, which will supply facilities for work with such materials as should be excluded from the general laboratory air. Each hood has a separate flue extending up into the attic and connecting with the main trunks in the exhaust, so that ample draft will be provided. The hoods are supplied with gas, water, vacuum, and sinks.

An exception to the general arrangements outlined above is found in the room on the ground floor devoted to the preparation of culture media. In this there is provided steam for use in connection with the autoclaves and sterilizers, as well as gas, water, and vacuum. The main autoclaves of the building, for the use of the biological laboratory, are placed in this room in which all of the culture media is prepared. It has the usual window desk and hood, but in place of the central work desk there is provided a heavy, square table intended to serve in preparing the various media.

The rooms for the botanists are not provided with the side tables and hoods. In their place the glass-front herbarium cases are mounted one above the other.

On each floor of the biological wing there is a room 10 feet wide and 24 feet long for the purpose of accommodating the refrigerating boxes and the incubators. The former are cooled by coils provided from the cold-storage equipment and are built in two sections, so that one portion may be kept at any temperature within the capacity of the plant, whereas the other may be regulated at 20° to 25°. The incubators are in the form of large boxes with a central door and shelves around the sides; they are 7 feet long and 3 feet wide, and are heated by Bunsen burners. Electric heat for the incubators is planned to be installed in the future, but it can not be operated successfully until after the hospital buildings are constructed, because it is not intended to run the power plant day and night until the necessity arises. The incubator rooms also have in them, attached to the walls, the smaller thermostats for heating paraffin for sectioning and smaller incubators for varying temperatures.

It is not intended to have any great number of smaller animals for experimental purposes inside of the building, and for convenience the house for guinea pigs and rabbits has been placed immediately to the rear of the biological wing. It consists of two large rooms, together with two smaller ones for operating, and a vestibule. One of the large rooms is to be devoted to the storage of animals and to the keeping of those which are under observation but not infected with dangerous diseases. The other large room is completely isolated and screened and is intended for work with plague, smallpox, cholera, and other diseases which may become dangerous and which it is not safe to handle unless every precaution is taken.

On the ground floor of the biological wing one room is set aside for the mechanic. This is provided with power and a complete equipment of lathes, shapers, drills, grinders, and tools, so that the laboratories will be in a position to have their instruments made and repaired on the grounds.

The rooms of the chemical wing show much greater variations among themselves than do those of the biological portion, because successful chemical work needs a greater variety of apparatus and facilities. However the distinction between the biological and chemical laboratories of the present time is not so great as it formerly was, because biologists are now carrying their investigations into fields more closely allied to chemistry. Therefore the general desk arrangement of the chemical rooms is similar to that of those devoted to biology. They each contain a window desk, which is 36 inches high and 30 inches wide, built without the central small sinks but in their place equipped with a trough, sink, drip board, gas, water, and vacuum for general chemical work.

In some of the rooms, especially those devoted to mineral analysis, sugar and foods, etc., one end of this window desk is left free without incumbrance of any kind, for the purpose of permanently placing burettes for making titrations. The central large laboratory desk is placed in most of the chemical rooms just as it is in the biological laboratory, with this difference, that it has two central troughs connected with the large sink, is equipped with a reagent shelf in the middle, and is provided with the usual connections throughout its length for compressed air, water, and vacuum.¹

The hoods in the chemical laboratory are of necessity much larger than they are in those portions of the building devoted to other work, some of them being 9 and others 12 and even 15 feet in length. The size of the flue for each hood is calculated so as to equalize in all the time necessary to effect a change of air; throughout the building they are provided with rising sash and glass fronts and sides.²

All the hoods in the chemical wing have not only gas, water, and vacuum but also steam and steam-exhaust pipes, so that evaporations may be continuously carried on without vitiating the air of the rooms. The wall tables opposite the hoods are equipped much in the same manner as are those in the biological wing, but a number of them, in rooms where this is necessary, have pipes for air pressure and steam as well. Storage-battery connections are found on the desks of the rooms devoted to mineral analysis, physics, and weights and measures, spectrum analysis, and in the private laboratory of the Superintendent. It is intended to utilize electrolytic methods of analysis to the fullest extent when the building is in complete operation, and as in this class of work it is necessary not infrequently, owing to acid fumes, to have the apparatus set up outside of the general laboratory air, these connections were also placed in the hoods.

Certain rooms devoted to specific purposes vary somewhat from the general type. On the ground floor entering from the east the first one to the right is devoted to physics and physical chemistry. It is provided with a large central pier with cement posts above

¹ Pl. XIII is a photograph of the central desk in the chemical laboratory and Pl. XIV of the same class in the biological laboratory.

² Pl. XV is a photograph of one of the larger hoods in the chemical laboratory.

and a top 8 inches thick, built upon a foundation which extends 3 feet below the level of the ground, provided with a broad footing and carried up entirely clear of the building. At a distance of 10 feet from the main pier and on each side is a secondary one brought up from the ground in the same manner, the group of three forming a triangle. All of the stability necessary for electric and other work is obtained by this arrangement. On one side of the main physics room is an ordinary wall desk supplied with gas and water for the purpose of electric conductivity measurements and physical chemistry. It is provided with storage-battery connection and is to have a large permanent thermostat tank attached to one end. Back of this main room to the east and connected with it by a doorway is a second one. It contains the ordinary wall desk and hood and is intended for the usual chemical work so necessary in a physical laboratory. Finally, to the east of the two just mentioned is a third for photometric work. It contains a long and heavy photometric table placed upon piers built in the same manner as the ones which have been described. The facilities in the division of weights, measures, and physics will be such that all classes of work needed in the adjustment of the weights and measures of the Islands can be carried on.

Across the hall from the rooms intended for physics and weights and measures is a large room to be used as a place for studying commercial processes. It has the usual central worktable and a long hood. One of the walls, differing from the general usage in the building, is occupied by a table on which are placed the shaking and stirring machines necessary for much of the work to be done in connection with the extraction of organic products. These machines are operated by a motor of one-half horsepower and are belt driven. The window desk is intended for distillations in which ether and other volatile inflammable substances are used, and as a consequence gas has been omitted from it, the heating being by means of steam only. At the east end is an alcove in which are placed various machines to be used in studying commercial processes. They consist of a copper still of 90 liters' capacity which is supplied with a steam jacket so arranged that either indirect or direct steam may be used. The apparatus is supplied with

¹ Pl. XVII is a photograph of the main room devoted to physics and physical chemistry.

vacuum connections, so that materials may be distilled in vacuo as well as under atmospheric pressure, and a double stopcock for the extraction of samples without interfering with the vacuum is attached. Next to this still stands a large extraction apparatus consisting of a boiler of 140 liters' capacity for the solvent. From this and extending to the extreme top is the exit tube connecting with the condenser, which is at the highest point; below this and between it and the still is mounted a receiving drum for the condensed solvents, and farther down and to one side is the extraction apparatus proper. This consists of a large kettle of 100 liters' capacity, steam jacketed to use heat if necessary, containing baskets for the placing of materials, and mounted on trunnions so that it may be tilted to empty and clean it. The entire apparatus is fitted with vacuum which is connected with the condenser above and the trunnions are so arranged that steam may be admitted through them into the extractor, which is fitted with the necessary safety valves to prevent accidents. A smaller still of 15 liters' capacity, on the same model as the large one, is also provided. of this apparatus is of the make of Gustav Christ, of Berlin. In the alcove of this room besides the apparatus given above there are mounted a porcelain ball mill for the pulverizing of refractory materials and a drug mill for grinding, both operated by a one-halfhorsepower motor with shafting and belting, a hydraulic press and a vacuum drying apparatus, the latter of the make of the American Vacuum Drving Company.1

Crossing the hall and immediately to the west of the room for physics is the assay laboratory. This is provided with a large firebrick table mounted on a pier and holding the main assay furnace from Braun & Co., Los Angeles, Cal., supplied with Cary hydrocarbon burners, the gasoline tank being placed in the ground outside the building so as to avoid all danger from fire. On each side of the main furnace are smaller ones, both muffle and crucible, of the Hoskins type. The main assay flue extends from these up to the roof and 15 feet above the latter. The corner of the assay room next the furnaces is occupied by a Braun crusher and pulverizer and a Bonnot ball mill, both operated by a 1-horsepower motor with shafting and belt. In the center of the room is a pier for the rolls and a large table for general work with samples. The

¹ Pl. XVIII is a photograph of the commercial apparatus described above.

other arrangements are like those of the chemical laboratory in general.1

Next to the assay room is a balance room for the general use of the ground floor and for the button balances, and leading from this is the combustion room for organic ultimate analysis, containing two combustion tables and one side table supplied with gas and water for the bomb furnaces.

The storerooms are two in number and are provided with shelving extending from the ground to the ceiling. One room is intended for chemicals and the other for apparatus, the former being supplied with a small laboratory worktable and hood and a distilling apparatus connected with the main boilers and to be used for the purpose of making distilled water.

In the chemical wing on the second floor the only variations from the general type are found in the room to the east and farthest along the corridor, in which is placed a special desk with compartments for the polariscopes, and in the main balance room. The latter has one central pier running from the ground up and constructed in the same manner as the ones described above. Upon this is placed a heavy balance table capable of accommodating eight instruments.²

The photographer's room on the second floor immediately to the left of the main staircase is fitted with two dark rooms 8 by 10 feet with large leaded sinks, drip board, shelving, and tables, and on one side is the stand for the photomicrographic apparatus. This is built on a solid pier 10 feet long and 30 inches wide, constructed on the same principle as the others in the building and placed well into the ground on broad footings. The photomicrographic apparatus is of the latest Zeiss pattern, both stand and camera, and the source of light is a 90° Thompson are light. The photographer is also supplied with 5 by 7 Graphic cameras with Zeiss series and A lenses, an 8 by 10 camera with Zeiss lens, as well as a Goertz anastigmat and enlarging camera and an apparatus for making lantern slides.³

The library consists of a main room connected on either side with the stack rooms. The librarian's desk is placed in the center

¹ Pl. XIX is a photograph of the furnaces and apparatus in the assay room.

² Pl. XX is a photograph of the balance room.

³ Pl. XXI is a photograph of the photographer's studio.

with the various files on either side, the reading room is in the alcove, and the book stacks are arranged on either side in the wings of the room and in the stack rooms. They are metal and of the type supplied by the Library Supply Company of Boston.¹

The serum laboratory in the power house has the same general arrangement of desks and hoods as the biological portion of the building. It needs no special description, with the exception of the serum kitchen, which contains a large, steam-supplied autoclave and sterilizer of the pattern made by Messrs. Bausch & Lomb, and a centrifugal from Lautenschlager, of Berlin, the latter driven by a four-horsepower motor. The serum laboratory also is provided with two large incubators of the same type as those used in the biological division. The vaccine stable is placed immediately next to the power house. It is fly-proof throughout, with cement floors and stalls for twenty calves and an operating room for inoculation and for collecting the virus. The calves in this place can be kept under thoroughly aseptic conditions. On the other side of the power house is located the building for small animals. The guinea pigs and rabbits will be kept in galvanized-iron cages placed on racks well in the center of the room to insure coolness. the rear of the driveway is a horse stable of a capacity sufficient to accommodate twelve horses, fly-proof, as in the case of the building for vaccine calves. It contains an operating room which will make it possible to collect serums according to the most improved methods. The last building of all is a small, two-storied one for dogs, goats, and monkeys.

APPARATUS AND SUPPLIES.

In Manila the greatest difficulty encountered by a laboratory is to keep on hand a sufficiency of special supplies, and also to provide the apparatus which may be necessary. While the equipment may apparently be complete, nevertheless requests for new classes of work may suddenly bring the laboratories face to face with the necessity of purchasing new kinds of apparatus, and until these are delivered of refusing to do the work demanded. To procure stores from Europe or America takes at least seven months. As a result the equipment of a laboratory at such a distance from the base of supplies must be somewhat more extensive and complete

¹ Pls. XXII-XXVI are photographs of the library showing the metal stacks.

than it would be in Western countries, because when the emergency arises it is not possible immediately to procure the materials necessary for the work.

A large portion of the special apparatus has been mentioned in the course of the description of the building, so that only a brief review of the remaining equipment will be necessary. The laboratory is supplied with fifteen microscopes of the best pattern from Zeiss, of Jena, and with two microscopes for travelers from Leitz. All the necessary lenses to equip these instruments for ordinary work are at hand, and in addition there are a certain number of apochromatic 2-millimeter aperture 1.30 and 1.40 lenses. Ocular and stage micrometers, Abbé drawing cameras, and other accessories are of necessity a part of the equipment. The microtomes were bought from Schanze, of Berlin, and are five in number, besides which there are two Minot automatic instruments and one using carbon dioxide for work with frozen sections.

The incubators are of the manufacture of F. & M. Lautenschlager and are divided into two classes—the portable ones for the individual rooms and the larger ones. All the necessary apparatus for work in preparing sections is also at hand.

The museum is amply supplied with jars of all sizes, and the storerooms contain the necessary surgical instruments, post-mortem sets, and other appliances necessary for biological work.

The chemical balances are of the make of Sartorius, of Goettingen, and of Rueprecht, of Vienna. There is one precision balance weighing to five kilograms and another to ten, to be used in the standardizing of weights and measures. Both of these are of the make of Sartorius. The laboratory has also a normal kilogram and a normal set of weights from 500 grams downward from Rueprecht, and a chief normal thermometer from A. Haak, of Jena, from whom all of the laboratory thermometers have been purchased. It is also provided with a complete set of normal specific-gravity apparatus, alcoholimeters, and other appliances for work with specific gravity. Apparatus for electric conductivity measurements from Goetze, of Leipsic, has been purchased, as well as normal volume measures from the same firm. An electric furnace for 300 amperes current for obtaining high temperatures is also a part of the chemical equipment. The necessary routine apparatus, such as flasks, beakers, Petrie dishes, evaporating dishes, platinum ware, retort stands, lamps, filtering apparatus, vacuum

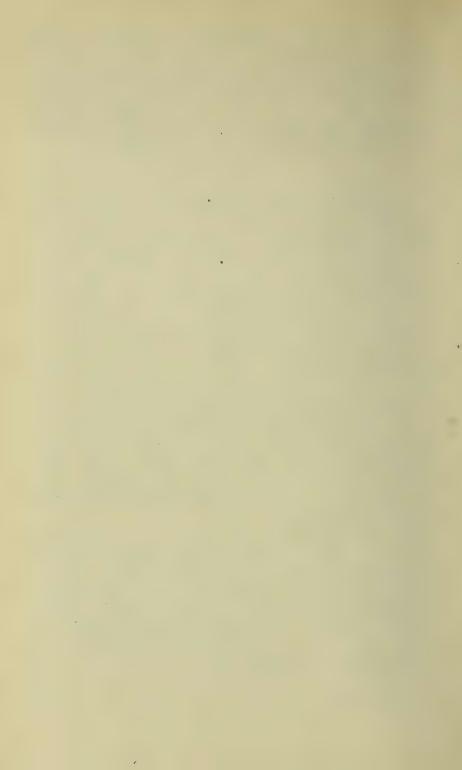
distilling flasks, condensers, etc., have been bought in sufficient quantity to meet all probable demands for the year, so that the laboratories are well equipped to meet emergencies. A detailed list of all the apparatus in the building would be too extensive for the present paper, nor would it be necessary, because the outline given above is sufficient to indicate the nature of the equipment.

CONCLUSION.

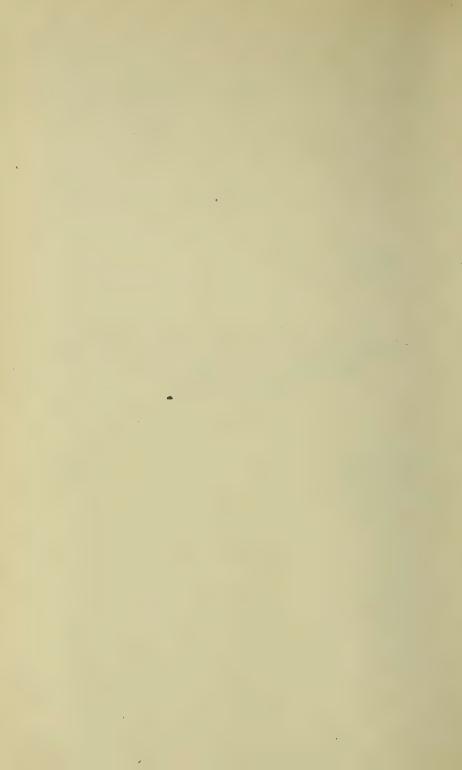
The Bureau of Government Laboratories, as the description given above will indicate, is now prepared to do all necessary work, and also to extend its field in the lines of investigation so essential for the success of any institution. The results of these investigations are being issued in a series of publications numbering up to the present time twenty-two. The list of these is given on the inside cover pages of this bulletin, and the range and variety of the work can best be judged by a perusal of the titles. One aim of the Bureau from the beginning has been to place itself in a position to attract to it foreign guests and students who may wish to come to Manila for the purpose of carrying on research work in which they are interested. To such investigators the doors of the laboratories will be open and the facilities of the Bureau will be at their disposal.

The scope of the library, as is shown by the librarian's description which follows, is such that no one need fear a lack of literature if he choose to carry on work in Manila. The Bureau, in the event that foreign investigators avail themselves of the privileges of the institution, will reserve to itself only the right of publishing their results as laboratory publications, however, without thereby in any way interfering with the desire of the investigators to send their work to the usual journals.

The Civil Government has given what it can to establish the Bureau on a proper basis. It has been most liberal in its support and in the encouragement of scientific work. However, it would seem as if an organization like the Bureau of Government Laboratories should also command the support of disinterested persons who wish to assist in the advance of science in general and in the Tropics in particular, and it is believed that the institution can be commended to such as one worthy of donations not only for specific but also for general purposes.



II. A CATALOGUE OF THE LIBRARY OF THE BUREAU OF GOVERNMENT LABORATORIES.



LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., February 14, 1905.

SIR: I have the honor to forward herewith, for publication as Part II of Bulletin No. 22, a catalogue of the Library of the Bureau of Government Laboratories, by Miss Mary Polk, librarian. It was thought that this catalogue would have been ready for publication in October, 1904, but, owing to the transfer during this month of 4,912 volumes from the other Bureaus of the Department of the Interior to the library of this Bureau, its issue has been delayed until the present time.

Very respectfully,

RICHARD P. STRONG,

Director Biological Laboratory,

Acting Superintendent of Government Laboratories.

Hon. DEAN C. Worcester,

Secretary of the Interior, Manila, P. I.

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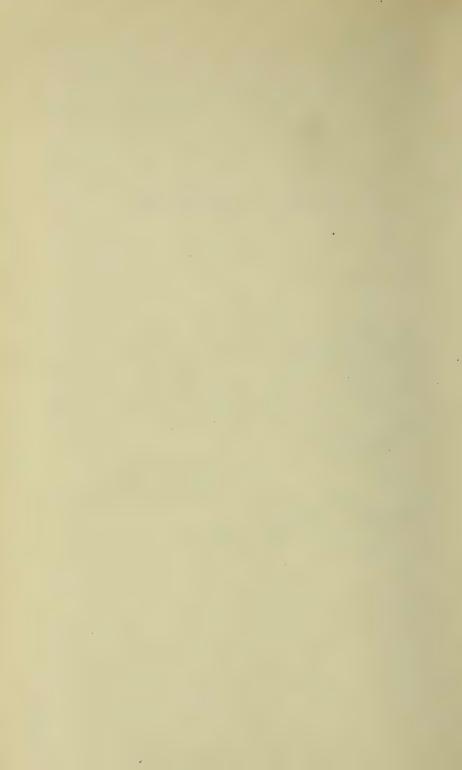


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Diagnosis, therapeutics, pharmacology, toxicology, medical jurisprudence, etc	
Pathology and bacteriology	
Surgery, dentistry, gynecology, and obstetrics	
Comparative and veterinary medicine	
Microscopy	
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Department of the Interior	
Bureau of Public Health	
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Anatomy and physiology	
Pharmacy and chemistry	
Diagnosis, therapeutics, and surgery	
Hygiene and sanitation	
Pathology and bacteriology	
Miscellaneous	
Public Health and Marine-Hospital Service	
Reference books	
Anatomy and physiology	
Diagnosis, therapeutics, and surgery	
Sanitation	
Pathology and bacteriology	
Philippine Civil Hospital.	
Bureau of Public Lands.	
Reference books	
General civil law	
Land and mining laws	
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Periodicals	
Agricultural methods and products.	
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PART II.

A CATALOGUE OF THE LIBRARY OF THE BUREAU OF GOVERNMENT LABORATORIES.

By MARY POLK, Librarian.

INTRODUCTION.

The following is a brief sketch of the organization, growth, and present condition of the library of the Bureau of Government Laboratories:

ESTABLISHMENT.

Provision for a scientific reference library as a part of the Bureau of Government Laboratories, Manila, P. I., was made in Act No. 156 of the United States Philippine Commission passed July 1, 1901. The Superintendent of Government Laboratories was given charge over the purchase of books authorized for use in connection with all Government laboratories for the Philippine Islands and was asked to prepare and recommend, as a basis for appropriation, to the central legislative body of the Islands—

- (a) Plans for a suitable building for the installation of the biological laboratory, the chemical laboratory, and a reference library.
 - (b) * * * (c) * * *
- (d) Detailed estimates of the cost of properly equipping the several laboratories and of procuring an adequate reference library, which shall be housed in the same building with the biological and chemical laboratories.

As the result of the presentation of such estimates, an agreement was entered into by which the library should receive the sum of \$45,290.66, United States currency, in quarterly, semiannual, or

annual installments, as best suited conditions existing at the times when appropriations were asked for. Act No. 389 of April 12, 1902, provided for the purchase of books not to exceed \$7,715.11, United States currency, and the first order under this appropriation was forwarded through the Insular Purchasing Agent May 21, 1902.

GROWTH AND PRESENT SIZE.

The nucleus of the library was formed by the transfer of about fifty books from the Board of Health and subscriptions for scientific periodicals for 1902, the latter beginning to arrive about July 1, 1902. The care of this material was so slight that the clerks of the Bureau took charge of it until the beginning of the year 1903, at which time the receipt of a large number of sets of periodicals and separate books and manuals, ordered during 1902, and the constantly growing list of subscriptions to current periodicals made it necessary for some one to assume the work of accessioning, classifying, cataloguing, and caring for the rapidly growing stock of books, periodicals, and pamphlets, and the writer was appointed librarian April 1, 1903.

The growth of the library has been steady, large orders being sent out two or three times during each year, and although the receipts have been delayed by long distance from the source of supply and the difficulty of finding in the market many of the sets of scientific periodicals ordered, the number of volumes on hand September 30, 1904, was 11,021, with outstanding orders for more than 70 sets of periodicals and about 250 other publications. Since that date 4,902 volumes have been added by transfer, 1,062 by purchase, and nearly 400, not previously included, by subscriptions for 1904, making a total number of more than 17,350 volumes on hand.

CHARACTER OF WORKS.

All works in the library are of a scientific character and represent the literature of the departments and divisions at present organized in the Bureau of Government Laboratories. The subscriptions to periodicals number about 250 and include many of the American, British, and continental chemical, biological, zoological, entomological, botanical, medical, photographic, library, microscopic, and general scientific periodicals, and the larger number of these have been on file since the establishment of the laboratories, the complete

sets being filled in as rapidly as funds become available. It has been the policy of the library to secure complete sets of journals in as many instances as possible in order that records of the history and development of scientific research may be available for reference by the investigators and for the routine work of the Bureau. The latest editions of scientific books and manuals have also been added, covering the work of the various divisions of the laboratory, and the list of dictionaries and general reference books includes English, German, French, Spanish, Italian, Dutch, Russian, Latin, and Greek dictionaries; the Index Medicus, complete; the Index Catalogue of the Surgeon-General's Library (Washington, D. C.), complete; Gould's Medical Dictionary and Encyclopedia; United States Dispensatory: Lippincott's Pronouncing Gazetteer; Polk's Medical Directory: Minerva; Roget's Thesaurus; International Catalogue of Scientific Literature; Reference Handbook of the Medical Sciences, etc.

The library has been augmented by the transfer of a number of books from other Bureaus. With the transfer of the Government botanist from the Bureau of Agriculture and the Bureau of Forestry to the Bureau of Government Laboratories a valuable collection of botanical books was added to the library. The Board of Health also added a number of volumes when the serum laboratory was transferred to this Bureau. For account of further transfers, see "Organization of a central scientific library" and "Supplemental list."

USE OF THE LIBRARY.

The legislation establishing the library as a part of the Bureau of Government Laboratories specified that it should be a reference library, and from the beginning the books have been on open shelves accessible for consultation by visitors. Lists of daily accessions have been available for reference, and as rapidly as possible shelf list and other helps in locating books on the shelves are being prepared. There is now adequate table room for readers, and attendants will furnish any book in the library on request.

As the work became better known it was found that there was a growing demand for books to be taken from the library, and at the beginning of the year 1904 arrangements were completed by which all persons employed in the various Departments of the Civil Government might take books on borrowers' cards, subject to the provisions of the following library rules which went into effect January 1, 1904:

LIBRARY RULES.

The library of the Bureau of Government Laboratories is primarily a reference library. These library rules have been prepared so as to enable the Bureau to accommodate employees of other Bureaus needing scientific literature, but in order not to lose the reference character of the library it is necessary that the following library rules be rigidly enforced and exactly complied with:

RULE I. The library shall be open daily from 10 to 12 and from 3.30 to 5.30 for the purpose of loaning books, excepting on Sundays and holidays. On Saturdays it will be open for this purpose from 10 to 12 only. The library shall be open for reading purposes from 8 to 12 and from 2.30 to 5.30 on week days, from 8 to 12 on Saturdays, and from 9 to 12 on Sundays. It will be closed on holidays.

Rule II. The privilege of taking books, periodicals, and pamphlets from the library shall be restricted to employees of the Civil Government: Provided, That each employee who wishes to take books, periodicals, or pamphlets from the library shall present to the librarian on the first occasion when such employee draws a book, periodical, or pamphlet a certificate from the chief of his Bureau stating that the person desiring to draw books is a civil employee and in the opinion of such chief of Bureau is a proper person to have the privilege of drawing books, and that such chief of Bureau accedes to and is willing to enforce the library rules herein laid down. The employee shall also sign a statement that he is willing to adhere to and abide by such library rules as are herein laid down.

RULE III. No person shall take from the library more than two books, three numbers of current periodicals, and five pamphlets at any one time, and each person so taking books, periodicals, or pamphlets shall sign a receipt to the librarian, on a form provided for the purpose, for each and every book, periodical, or pamphlet drawn.

RULE IV. Books and pamphlets shall not be retained for a period greater than fourteen days; monthly periodicals and those appearing at less frequent intervals not more than five days.

Rule V. Any book, periodical, or pamphlet taken from the library shall be promptly returned to the library upon notice from the librarian that such book, periodical, or pamphlet is needed in the library, even though the time limit for keeping such book, periodical, or pamphlet may not have expired.

Rule VI. All books, periodicals, or pamphlets shall be returned directly to the librarian by the borrower, and under no circumstances shall they be returned to the shelf by the borrower.

RULE VII. Books, periodicals, or pamphlets used at the reading table shall be left on the table and not returned to the shelves by the borrower.

RULE VIII. The loan of any book, periodical, or pamphlet may be

renewed by any civil employee upon request to the librarian at the end of the time specified above under Rule IV: *Provided always*, That no demand for such book, periodical, or pamphlet shall have been made upon the librarian during the period in which the book has been out of the library.

RULE IX. By and with the consent of the Superintendent of Government Laboratories, a certain number of books needed for frequent reference may be withdrawn from circulation and retained upon the shelves of the library, such list to be revised and altered from time to time, as deemed expedient.

RULE X. Any civil employee retaining a book, periodical, or pamphlet beyond the time specified in Rule IV, provided the loan be not renewed to him by the librarian, will be subject to a fine of one peso (P1), Philippine currency, for each book, periodical, or pamphlet so retained by him for each three days or fraction thereof over and above the time specified in Rule IV during which the book, periodical, or pamphlet has been retained.

RULE XI. Every civil employee borrowing books, periodicals, or pamphlets from the library shall be responsible for any damage to the same or for the loss of the same, and charges for such loss or damage shall be assessed by the librarian as follows:

- (a) For loss or destruction.—For each text-book, manual, or other single volume, and for each volume not older than three years belonging to a set, twice its value as advertised by the publisher or as shown by the bills paid, and for each volume older than three years belonging to a set, five times its value as advertised by the publisher or as shown by the bills paid.
- (b) Loss by damage.—All loss by damage to books, periodicals, or pamphlets shall be assessed by the librarian and charged accordingly: Provided, however, That if, in the opinion of the librarian, the damage is such as to require the purchase of a new book, then the charge shall be assessed as in class (a) of this rule.

RULE XII. All moneys due to the Bureau of Government Laboratories by reason of the application of the foregoing rules, if not paid within two weeks after sending of notice from the librarian, shall be recovered through the disbursing officer of the Bureau to which the civil employee belongs, by application by the Superintendent of Government Laboratories for such moneys.

RULE XIII. The privilege of borrowing books from the library by any civil employee may be withdrawn by the Superintendent of Government Laboratories if the latter is satisfied that such civil employee is not adhering to library rules, or is careless in the handling of books.

Rule XIV. The provisions of the foregoing rules apply to civil employees stationed in Manila. Under no circumstances will books be loaned for use outside of Manila.

LOCATION.

The library is now permanently located in the new Government Laboratory, Calle Herran, and occupies the middle rooms in the front of the building, having a floor space 86 by 24 feet, with an extension 26 by 13 feet in the center, in which are placed the shelves for current numbers of periodicals, map and chart case, and reading tables. Two side rooms each 24 by 16 feet and three alcoves each approximately 13 feet square contain book stacks of modern construction, entirely of metal with adjustable wooden shelves 8 and 10 inches wide. The main central room contains the librarian's desk, two sixty-drawer card cabinet files, and two revolving bookcases. The space in front of these is left open and can be utilized excellently for scientific meetings. ¹

CLASSIFICATION AND CATALOGUING.

From the beginning a catalogue of books by author and title, of periodicals by titles alphabetically arranged, a business record of books ordered and received, and an accession book for periodicals have been kept. An accession book for all additions to the library is partially compiled and when completed will give complete data concerning all the books and periodicals.

The books and periodicals are arranged on the shelves according to the Dewey system of classification very slightly modified, and a shelf list is arranged for a portion of the library. This shelf list, numbered and arranged by the Dewey classification method, is at the same time a subject catalogue of the library, all publications on a certain subject coming under the same number. For example, all works on general science fall under 500, those on chemistry under 540, those on medicine under 610, those on photography under 770, etc. The books will be made still easier of access by placing in each one and on every card referring to it a combination of four numbers which will show the exact shelf on which the book is located. For example, "1/10/3/6" placed in a book and on a card means that this volume may be found in room (or alcove) No. 1, on stack face No. 10, division No. 3, and shelf No. 6, counting from the floor. This method tells the reader at a glance on which 3-foot shelf in the library the volume desired is located.

No subject or dictionary catalogue has as yet been inaugurated, this being an impossibility until more assistants have been employed and time taken for their training.

¹ See floor plan and photographs.

EXCHANGES.

The library receives continually a large number of exchanges from scientific institutions, many as monographs and others in periodical form. Where these are complete publications, or if periodical, when the current numbers are received regularly, they have been listed in this catalogue. As rapidly as these sets are completed they will be published in supplementary lists.

CATALOGUES.

The library has collected from time to time a large number of catalogues of (1) leading American colleges and universities, (2) leading manufacturers of laboratory and scientific apparatus and supplies, and (3) American, British, and continental book dealers and publishers. These are indexed by the card system and where unbound are protected by pamphlet cases. They are at all times available for use in the reference room and for circulation under the regular library rules.

MAPS AND CHARTS.

A number of charts of harbor and coast lines of Philippine waters issued by the Coast and Geodetic Survey, and maps published by the Military Information Division, Office of the Chief of Staff, War Department, Washington, D. C., and Manila, P. I., have been presented to the library during the past two years, but until recently no adequate way of caring for them had been arranged. A large native hard-wood case containing eight drawers 4 by 5 feet in size and 2½ inches deep, lately placed in the reading room, provides an excellent place for caring for and consulting this class of material. An effort will be made to secure other maps and charts, especially those of local interest, and a list will be published when the collection is more complete.

GOVERNMENT PUBLICATIONS.

Some 3,500 Government publications, including those of various Departments of the United States and of the Bureaus of the Philippine Government, as well as many foreign government reports, are on file and are indexed by the card system according to title and department number under which they are published. It is desired to complete many of these sets before including them by

title in a printed list, as many sets have numbers missing which we hope it may be possible to secure by further correspondence.

ORGANIZATION OF A CENTRAL SCIENTIFIC LIBRARY.

On October 20, 1904, at the call of the honorable the Secretary of the Interior, a meeting of the chiefs of the various Bureaus of the Department of the Interior was held in the reading room of the library and plans completed for establishing a central card catalogue for all scientific publications belonging to the various Bureaus of this Department. All books and periodicals at that time in these different Bureaus were to be transferred to the Bureau of Government Laboratories, where accurate date for publishing a printed catalogue and for inaugurating a comprehensive card catalogue should be collected, after which all works needed for constant reference in the various offices could be returned to the individual Bureaus on memorandum receipt signed by the chief of the Bureau and approved by the Secretary of the Interior.

Resulting from this arrangement 4,902 volumes have been transferred to the Bureau of Government Laboratories and information cards made from them containing data for issuing a printed bulletin, for entering these titles in the library accession book, and for establishing an author-title card index, each card referring to any one of these publications showing in what Bureau or Office the volume may be found if it has been taken from the library on memorandum receipt. These works have all been included by title in the present bulletin, except in the case of a considerable amount of miscellaneous material similar to that discussed under "Exchanges" and "Government publications" above, received from the Bureau of Public Health, the Public Health and Marine-Hospital Service, the Philippine Civil Hospital, the Bureau of Public Lands, the Bureau of Agriculture, the Mining Bureau, the Bureau of Forestry and the Ethnological Survey.

In the collection and classification of the material for these supplemental lists much valuable assistance has been given by Mr. C. J. Arnell, stenographer of the biological laboratory.

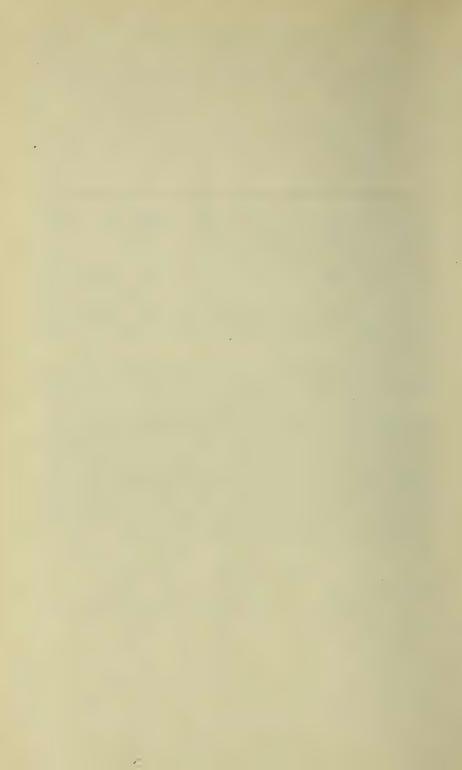
Throughout this catalogue titles have been quoted in the language of the publication as far as possible. In a very few cases titles in Japanese, Russian, and Hindustani have been translated into English and one Greek subtitle into Latin. In the case of the Japanese publications transliteration of the original title has also been given.

In the following periodical lists a number of publications not ordinarily classed as periodicals have been included. All serial publications are carried on the business records of the library as periodicals, and many of these are used in such a way that it has not seemed well to make a division here, hence all have been included. Practically all titles of serial works appear also in the lists of books under their appropriate subject classifications.

Certain journals are frequently called for by the name of the editor—e. g., Koch's Jahresbericht, Grafe's Archiv, Hoppe-Seyler's Zeitschrift, etc. In the alphabetical lists these have original entries which follow title page of periodical, with cross references to other forms.

For convenience in consulting periodical lists two arrangements, one alphabetical and the other by subject, have been included in this catalogue. In lists arranged by subject the titles appear according to the language (or country) in which the periodical is published, in the following order: American, English, German, French, other languages (or countries). Publications of learned societies follow regular periodical publications. Cross references are not considered necessary in lists arranged by subject.

In lists of complete sets of periodicals both inclusive volume numbers and inclusive years of publication are considered worth giving, since references to literature are far from uniform in this respect.



LISTS.

LIST OF CURRENT PERIODICALS ARRANGED ALPHABETICALLY.

Allgemeine botanische Zeitschrift, Karlsruhe, 8°.

Allgemeine botanische Zeitung. See Flora.

American Annual of Photography and Photographic Times-Bulletin Almanac, New York, 8°.

American Chemical Journal, Baltimore, 8°.

American Journal of the Medical Sciences, Philadelphia and New York, 8°.

American Journal of Physiology, Boston, 8°.

American Journal of Science (Silliman), New Haven, 8°.

American Medicine, Philadelphia, 4°.

American Veterinary Review, New York, 8°.

Anatomische Hefte (Merkel und Bonnet. Beiträge und Referate zur Anatomie und Entwicklungsgeschichte, part 1), Wiesbaden, 8°.

Anatomischer Anzeiger, Jena, 8°.

Annalen der Chemie. See Liebig's Annalen der Chemie.

Annalen der Physik, Leipzig, 8°.

Annales de chimie et de physique, Paris, 8°.

Annales de l'Institut Pasteur, Paris, 8°.

Annales de la Société entomologique de Belgique, Bruxelles, 8°.

Annales de la Société entomologique de France, Paris, 8°.

Annales des sciences naturelles, Botanique, Paris, 8°.

Annales du Jardin botanique de Buitenzorg, Leide, 8°.

Annali d'igiene sperimentale, Milano-Torino-Roma-Napoli, 8°.

Annals of Botany (Balfour), London, 8°.

Annals of the Royal Botanic Garden, Calcutta, 4°.

Annotationes zoologicae Japonenses (Nihon Dobutsugaku Iho), Tokyo, 8°.

Arbeiten aus dem kaiserlichen Gesundheitsamte, Berlin, 8°.

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Archiv der Pharmacie, Berlin, 8°.

Archiv für Anatomie und Physiologie (Anatomische Abtheilung= Archiv für Anatomie und Entwicklungsgeschichte; Physiologische Abtheilung= Archiv für Physiologie), Leipzig, 8°.

Archiv für Dermatologie und Syphilis, Wien und Leipzig, 8°.

Archiv für experimentelle Pathologie und Pharmakologie, Leipzig, 8°.

Archiv für Hygiene, München und Berlin, 8°.

Archiv für klinische Chirurgie (Langenbeck), Berlin, 8°.

Archiv für mikroskopische Anatomie und Entwicklungsgeschichte, Bonn, 8°.

Archiv für Ophthalmologie. See Graefe's Archiv.

Archiv für pathologische Anatomie, etc. See Virchow's Archiv.

Archiv für Schiffs- und Tropen-Hygiene (Mense), Leipzig, 8°.

Archiv für Verdauungskrankheiten, Berlin, 8°.

Archiv für wissenschaftliche und praktische Thierheilkunde, Berlin, 8°.

Archives d'anatomie microscopique, Paris, 8°.

Archives de biologie (Beneden et Bambeke), Liége et Paris, 8°.

Archives italiennes de biologie, Turin, 8°.

Archives générales de medecine, Paris, 8°.

Archives de médecine expérimentale et d'anatomie pathologique, Paris, 8°.

Archives de médecine navale, Paris, 8°.

Archives médicales de Toulouse, Toulouse et Paris, 8°.

Archives de parasitologie (Blanchard), Paris, 8°.

Archives des sciences biologiques, St.-Pétersbourg, 8°.

Archivio di biologia normale e patologica. See Sperimentale.

Archivio per le scienze mediche, Torino, 8°.

Association Medical Journal. See British Medical Journal.

Atlas of Illustrations of Clinical Medicine, Surgery and Pathology (New Sydenham Society), London, 4°.

Balfour's Annals of Botany. See Annals of Botany.

Baumgarten's Jahresbericht. See Jahresbericht...von den pathogenen Mikroorganismen.

Beihefte zum botanischen Centralblatt, Jena, 8°.

Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie (Ziegler), Jena, 8°.

Beneden et Bambeke's Archives. See Archives de biologie.

Berichte der deutschen botanischen Gesellschaft, Berlin, 8°.

Berichte der deutschen chemischen Gesellschaft, Berlin, 8°.

Berliner entomologische Zeitschrift, Berlin, 8°.

Berliner klinische Wochenschrift, Berlin, 4°.

Berliner tierärztliche Wochenschrift, Berlin, 4°.

Bibliographia zoologica. See Zoologischer Anzeiger.

Biedermann's Central-Blatt. See Central-Blatt für Agrikulturchemie.

Biochemisches Centralblatt, Leipzig, 8°.

Biological Bulletin of the Marine Biological Laboratory, Woods Holl, Mass., Lancaster, Pa., 8°.

Biologisches Centralblatt (Rosenthal), Leipzig, 8°.

Blanchard's Archives. See Archives de parasitologie.

Boston Medical and Surgical Journal, Boston, 4°.

Botanical Gazette, Chicago, 8°.

Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie (Engler), Leipzig, 8°.

Botanische Zeitung, Leipzig, 4°.

Botanischer Jahresbericht. See Just's Botanischer Jahresbericht.

Botanisches Centralblatt, Leiden, 8°.

British Journal of Dental Science, London, 8°.

British Medical Journal (Association Medical Journal), London, 8°.

Bulletin de l'Académie de médecine, Paris, 8°.

Bulletin de l'Herbier Boissier, Genève, 8°.

Bulletin de l'Institut Pasteur, Paris, 8°.

Bulletin de la Société chimique de Paris, 8°.

Bulletin de la Société entomologique, Paris, 8°.

Bulletin of the British Ornithologist's Club, London, 8°.

Bulletin of the Johns Hopkins Hospital, Baltimore, 4°.

Bulletin of the Torrey Botanical Club, Lancaster, Pa., 8°.

Bulletins of the Bureau of Government Laboratories, Manila, 8°.

Bulletins of the Hygienic Laboratory, Public Health and Marine-Hospital Service of the United States, Washington, 8°.

Bullettino delle scienze mediche, Bologna, 8°.

Canadian Entomologist, London, Ontario, 8°.

Carus' Anzeiger. See Zoologischer Anzeiger.

Central-Blatt für Agrikulturchemie (Biedermanns), Leipzig, 8°.

Centralblatt für allgemeine Pathologie und pathologische Anatomie (Ziegler), Jena, 8°.

Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, part 1, Originale and Referate, part 2, Jena, 8°.

Centralblatt für Gynäkologie, Leipzig, 8°.

Centralblatt für innere Medicin, Leipzig, 8°.

Centralblatt für die medicinischen Wissenschaften, Berlin, 8°.

Chemical News and Journal of Physical Science, London, 4°. (With no. 1, vol. 1, Chemical Gazette was incorporated with this journal.)

Chemische Industrie, Berlin, 4°.

Chemisches Central-Blatt, Berlin, 8°.

Comptes rendus . . . de l'Académie des sciences, Paris, 4°.

Comptes rendus . . . de la Société de biologie, Paris, $8^{\circ}.$

Contributions from the United States National Herbarium, Washington, 8°.

Correspondenz-Blatt für Zahnärzte, Berlin, 8°.

Curtis's Botanical Magazine, London, 8°.

De Dalla Torre et Harms' Genera siphonogamarum. See Genera siphonogamarum.

Dental Cosmos, Philadelphia, 8°.

Dental Era, St. Louis, 8°.

Dental Review, Chicago, 8°.

Deutsche medicinische Wochenschrift, Berlin, 4°.

Deutsche tierärztliche Wochenschrift, Hannover, 4°.

Deutsches Archiv für klinische Medicin, Leipzig, 8°.

Engineering and Mining Journal, New York, 4°.

Engler's Botanische Jahrbücher. See Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie.

Engler. Das Pflanzenreich. See Pflanzenreich.

Entomological News, Philadelphia, 8°.

Entomologische Zeitschrift, Guben, 4°.

Entomologisk Tidskrift, Stockholm, 8°.

Entomologist, London, 8°.

Entomologist's Monthly Magazine, London, 8°.

Ergebnisse der allgemeinen Pathologie (Lubarsch-Ostertag), Wiesbaden, 8°.

Exotische Käfer (Heyne), Leipzig, 4°.

Exotische Schmetterlinge (Hübner), Leipzig, 4°.

Experiment Station Record (United States Department of Agriculture), Washington, 8°.

Fischer's Jahres-Bericht. See Jahres Bericht der . . . chemischen Technologie.

Flora, oder Allgemeine botanische Zeitung, Marburg, 8°.

Gardeners' Chronicle. See Supplemental list, Bureau of Agriculture.

Gazette des hôpitaux, Paris, 4°.

Gazette hebdomadaire des sciences médicales de Bordeaux, 4°.

Gazette médicale de Paris, 4°.

Gazzetta chimica italiana, Roma, 8°.

Gazzetta medica di Roma, 8°.

Genera insectorum (Wytsman), Bruxelles, 4°.

Genera siphonogamarum (De Dalla Torre et Harms), Leipzig, 4°.

Giornale della reale Società ed Accademia veterinaria italiana, Torino, 8°.

Graefe's Archiv für Ophthalmologie, Leipzig, 8°.

Hare, Progressive Medicine. See Progressive Medicine.

Hedwigia. Organ für Kryptogamenkunde und Phytopathologie, Dresden, 8°.

Hermann's Jahresbericht. See Jahresbericht . . . der Physiologie. Heyne's Exotische Käfer. See Exotische Käfer.

Hooker's Icones plantarum, London, 8°.

Hoppe-Seyler's Zeitschrift für physiologische Chemie, Strassburg, 8°.

Hübner's Exotische Schmetterlinge. See Exotische Schmetterlinge. Hygienische Rundschau, Berlin, 8°.

Ibis, a Quarterly Journal of Ornithology, London, 8°.

Index Catalogue of the Library of the Surgeon-General's Office, United States Army, 2. series, Washington, 8°.

Index Kewensis plantarum phanerogamarum, 1. and 2. supplements, Brussels and Oxford, 4°.

Index Medicus, 2. series, Washington, 8°.

India Rubber World, New York, 4°.

Indian Forester. See Supplemental list, Bureau of Agriculture and Bureau of Forestry.

Indian Lancet, Calcutta, 4°.

Indian Medical Gazette, Calcutta, 4°.

International Catalogue of Scientific Literature, London, 8°.

International Clinics, Philadelphia, 8°.

International Dental Journal, Philadelphia, 8°.

Items of Interest, New York, 8°.

Jahrbuch der Chemie (Meyer), Braunschweig, 8°.

Jahrbücher der in- und ausländischen gesammten Medicin. See Schmidt's Jahrbücher.

Jahrbücher für wissenschaftliche Botanik (Pringsheim), Leipzig, 8°.

Jahresbericht der Pharmacie, Göttingen, 8°.

Jahresbericht über die Fortschritte der Agriculturchemie, Berlin, 8°.

Jahresbericht über die Fortschrite der Chemie und verwandten Teile anderer Wissenschaften (Liebig und Kopp), Braunschweig, 8°.

Jahresbericht über die Fortschritte in der Lehre von den Gärungs-Organismen (Koch), Leipzig, 8°.

Jahresbericht über die Fortschritte in der Lehre von den pathogenen Mikroorganismen (Baumgarten), Leipzig, 8°.

Jahresbericht über die Fortschritte der Physiologie (Hermann), Stuttgart, 8°.

Jahresbericht über die Fortschritte der Tier-Chemie, oder der physiologischen und pathologischen Chemie, Wiesbaden, 8°.

Jahresbericht über die Fortschritte und Leistungen auf dem Gebiete der Hygiene (Uffelmann), Braunschweig, 8°.

Jahres-Bericht über die Leistungen der chemischen Technologie (Fischer), Leipzig, 8°.

Jahresbericht über die Leistungen und Fortschritte in der gesammten Medicin (Waldeyer und Posner) Berlin, 8°. Continuation of Virchow und Hirsch's Jahresbericht.

Johns Hopkins Hospital Reports, Baltimore, 8°.

Journal de l'anatomie et de la physiologie normales et pathologiques, Paris, 8° .

Journal de botanique, Paris, 8°.

Journal d'hygiene, Paris, 4°.

Journal de médecine de Paris, 4°.

Journal de médecine vétérinaire et de zootechnie, Lyon, 8°.

Journal de physiologie et de pathologie générale, Paris, 8°.

Journal für praktische Chemie, Leipzig, 8°.

Journal of the American Chemical Society, Easton, Pa., 8°.

Journal of the American Medical Association, Chicago, 4°.

Journal of the Asiatic Society of Bengal, Calcutta, 8°.

Journal of Bacteriology (Saikingaku Zasshi), Tokyo, 8°.

Journal of Botany, British and Foreign, London, 8°.

Journal of the Chemical Society, London, 8°.

Journal of Comparative Pathology and Therapeutics, Edinburgh and London, 8°.

Journal of Cutaneous Diseases including Syphilis, New York, 8°.

Journal of Experimental Medicine, Baltimore, 8°.

Journal of Hygiene, Cambridge, 8°.

Journal of Infectious Diseases, Chicago, 8°.

Journal of the Linnean Society, Botany, London, 8°.

Journal of Medical Research, Boston, 8°.

Journal of Mycology, Columbus, Ohio, 8°.

Journal of Pathology and Bacteriology, Edinburgh and London, 8°.

Journal of the Royal Microscopical Society, London, 8°.

Journal of the Society of Chemical Industry, London, 8°.

Journal of Tropical Medicine, London, 8°.

Just's Botanischer Jahresbericht, Leipzig, 8°.

Kew Bulletin of Miscellaneous Information, London, 8°.

Klinisches Jahrbuch, Jena, 8°.

Koch's Jahresbericht. See Jahresbericht . . . von den Gährungs-Organismen.

Koch und Flügge's Zeitschrift. See Zeitschrift für Hygiene und Infectionskrankheiten.

Lancet, London, 4°.

Lancette française, Gazette des hôpitaux civils et militaires. See Gazette des hôpitaux.

Langenbeck's Archiv. See Archiv für klinische Chirurgie.

Library Journal, New York, 8°.

Liebig's Annalen der Chemie, Leipzig, 8°.

Liebig und Kopp's Jahresbericht. See Jahresbericht über die Fortschritte der Chemie, etc.

Lubarsch-Ostertag's Ergebnisse. See Ergebnisse der allgemeinen Pathologie.

Malpighia, Genova, 8°.

Mededeelingen uit 'sLands Plantentuin, Batavia, 8°.

Medical Library and Historical Journal, Brooklyn, N. Y., 8°.

Medical News, New York, 8°.

Medical Record, New York, 4°.

Medical Review, London, 8°.

Medical Review of Reviews, New York and London, 8°.

Memoirs from the Biological Laboratory of the Johns Hopkins University, Baltimore, 8°.

Mense's Archiv. See Archiv für Schiffs- und Tropen-Hygiene.

Meyer's Jahrbuch der Chemie. See Jahrbuch der Chemie.

Missouri Botanical Garden Reports, St Louis, 8°.

Mittheilungen aus der medicinischen Facultät der kaiserlich-japanischen Universität zu Tokio (Teikoku Daigaku Kiyo Iken), Tokyo, 8°.

Monatshefte für Chemie, Wien, 8°.

Monatshefte für praktische Dermatologie (Unna und Taenzer), Hamburg, 8°.

Montreal Medical Journal, Montreal, 8°.

Münchener medicinische Wochenschrift, München, 4°.

Nature, London, 4°.

New York Medical Journal and Philadelphia Medical Journal, New York, 4°.

Northwest Medicine, Seattle, Wash., 8°.

Nothnagel's Encyclopedia of Practical Medicine, Philadelphia and London, 8°.

Notizblatt des königl. botanischen Gartens und Museums zu Berlin, Leipzig, 8°.

Oesterreiche botanische Zeitschrift, Wien, 8°.

Oesterreiche Chemiker-Zeitung, Wien, 4°.

Oesterreiche Monatschrift für Tierheilkunde, Wien, 8°.

Pacific Dental Gazette, San Francisco, 8°.

Pennsylvania Medical Journal, Athens, Pa., 8°.

Pflanzenreich (Engler), Leipzig, 8°.

Philosophical Transactions of the Royal Society of London, 4°.

Photographic Times-Bulletin, New York, 8°.

Photographische Mitteilungen, Berlin, 8°.

Prager medicinische Wochenschrift, Prag, f°.

Presse médicale, Paris, f°.

Pringsheim's Jahrbücher. See Jahrbücher für wissenschaftliche Botanik.

Proceedings of the Entomological Society of Washington, 8°.

Proceedings of the Royal Society of London, London, 8°.

Progrès dentaire, Paris, 8°.

Progrès médical, Paris, 4°.

Progressive Medicine (Hare), Philadelphia and New York, 8°.

Public Health Reports (United States Public Health and Marine-Hospital Service), Washington, 8°.

Public Libraries, Chicago, 8°.

Quarterly Circular, London, 8°.

Quarterly Journal of Microscopical Science, London, 8°.

Records of the Botanical Survey of India, Calcutta, 8°.

Recueil de médecine vétérinaire, Paris, 8°.

Recueil des travaux chimiques des Pays-Bas et de la Belgique, Leide, 8°.

Revue d'entomologie, Caen, 8°.

Revue de médecine, Paris, 8°.

Revue mycologique, Toulouse, 8°.

Rockefeller Institute Studies. See Studies from the Rockefeller Institute for Medical Research.

Rosenthal's Centralblatt. See Biologisches Centralblatt.

Russkii Vratch, St. Petersburgh, 4°.

Sammlung chemischer und chemisch-technischer Vorträge (Ahrens), Stuttgart, 8°.

Schmidt's Jahrbücher der in- und ausländischen gesammten Medicin, Leipzig, 4°.

Schweizer-Archiv für Tierheilkunde, Zürich, 8°.

Science, a weekly journal devoted to the advancement of science publishing the official notices and proceedings of the American Association for the Advancement of Science, Lancaster, Pa., 4°.

Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, Calcutta, 4°.

Semaine médicale, Paris, 4°.

Silliman's Journal of Science. See American Journal of Science.

Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, mathematisch- naturwissenschaftliche Klasse, Wien, 8°.

Sperimentale = Archivio di biologia normale e patologica. Firenze, 8°.

Stettiner entomologische Zeitung, Stettin, 8°.

St. Louis and Canadian Photographer, St. Louis, 8°.

St. Petersburger medicinische Wochenschrift, St. Petersburg, 4°.

Stomatologiai Közlöny, Budapest, 8°.

Studies from the Rockefeller Institute for Medical Research, New York, 8°.

Therapeutic Gazette, Detroit, 8°.

Tierärztliche Rundschau, Friedenau-Berlin, 4°.

Tierärztliches Centralblatt, Wien, 8°.

Transactions of the American Entomological Society, Philadelphia, 4° .

Transactions of the American Microscopical Society, Lancaster, Pa., 8°.

Transactions of the Entomological Society of London, 8°.

Transactions of the Linnean Society, 2. series, Botany, London, 4° .

Uffelmann's Jahresbericht. See Jahresbericht...auf dem Gebiete der Hygiene.

Univ. of Penna. Medical Bulletin, Philadelphia, 4°.

Unna und Taenzer's Monatshefte. See Monatshefte für praktische Dermatologie.

Virchow's Archiv für pathologische Anatomie und Physiologie und für klinische Medicin, Berlin, 8°.

Virchow und Hirsch's Jahresbericht. See Jahresbericht. . . . der gasammten Medicin.

Waldeyer und Posner's Jahresbericht. See Jahresbericht...der gesammten Medicin.

Wiener entomologische Zeitung, Wien, 8°.

Wiener klinische Wochenschrift, Wien, 4°.

Wiener medicinische Wochenschrift, Wien, 4°.

Wytsman's Genera insectorum. See Genera insectorum.

Zeitschrift für analytische Chemie, Wiesbaden, 8°.

Zeitschrift für angewandte Chemie, Berlin, 4°.

Zeitschrift für angewandte Mikroskopie, Leipzig, 8°.

Zeitschrift für anorganische Chemie, Hamburg und Leipzig, 8°.

Zeitschrift für Biologie, München und Berlin, 8°.

Zeitschrift für Entomologie, Berlin, 8°.

Zeitschrift für Hygiene und Infectionskrankheiten (Koch und Flügge), Leipzig, 8°.

Zeitschrift für Hymenopterologie und Dipterologie, Teschendorf i. Mecklenburg, 8°.

Zeitschrift für Instrumentenkunde, Berlin, 8°.

Zeitschrift für klinische Medicin, Berlin, 8°.

Zeitschrift für öffentliche Chemie, Plauen i. V., 8°.

Zeitschrift für Pflanzenkrankheiten, Stuttgart, 8°.

Zeitschrift für physikalische Chemie, Leipzig, 8°.

Zeitschrift für physiologische Chemie. See Hoppe-Seyler's Zeitschrift für physiologische Chemie.

Zeitschrift für Thiermedicin, Jena, 8°.

Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, Berlin, 8°.

Zeitschrift für Veterinärkunde, Berlin, 8°.

Zeitschrift für wissenschaftliche Mikroskopie, Leipzig, 8°.

Zeitschrift für wissenschaftliche Zoologie, Leipzig, 8°.

Ziegler's Beiträge. See Beiträge zur pathologischen Anatomie, etc. Ziegler's Centralblatt. See Centralblatt für allgemeine Pathologie,

etc.

Zoological Record, London, 8°.

Zoologischer Anzeiger, Leipzig, 8°.

LIST OF CURRENT PERIODICALS ARRANGED BY SUBJECT.

GENERAL SCIENCE AND MICROSCOPY.

American Journal of Science (Silliman), New Haven, 8°.

International Catalogue of Scientific Literature, London, 8°.

Science, a weekly journal devoted to the advancement of science publishing the official notices and proceedings of the American Association for the Advancement of Science, Lancaster, Pa., 4°.

Journal of the Asiatic Society of Bengal, Calcutta, 8°.

Nature, London, 4°.

Zeitschrift für angewandte Mikroskopie, Leipzig, 8°.

Zeitschrift für wissenschaftliche Mikroskopie, Leipzig, 8°.

Transactions of the American Microscopical Society, Lancaster, Pa., 8°.

Journal of the Royal Microscopical Society, London, 8°.

Philosophical Transactions of the Royal Society of London, 4°.

Proceedings of the Royal Society of London, 8°.

Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Klasse, Wien, 8°.

Comptes rendus . . . de l'Académie des sciences, Paris, 4°.

PHYSICS AND CHEMISTRY.

Annalen der Physik, Leipzig, 8°.

Zeitschrift für Instrumentenkunde, Berlin, 8°.

Annales de chimie et de physique, Paris, 8°.

American Chemical Journal, Baltimore, 8°.

Chemical News and Journal of Physical Science, London, 4° (with no. 1, vol. 1, Chemical Gazette was incorporated with this journal).

Chemisches Central-Blatt, Berlin, 8°.

Jahrbuch der Chemie (Meyer), Braunschweig, 8°.

Jahresbericht über die Fortschritte der Chemie und verwandter Teile anderer Wissenschaften (Liebig und Kopp), Braunschweig, 8°.

Jahresbericht über die Fortschritte der Tier-Chemie oder der physiologischen und pathologischen Chemie, Wiesbaden, 8°.

Journal für praktische Chemie, Leipzig, 8°.

Liebig's Annalen der Chemie, Leipzig, 8°.

Monatshefte für Chemie, Wien, 8°.

Oesterreiche Chemiker-Zeitung, Wien, 4°.

Sammlung chemischer und chemisch-technischer Vorträge (Ahrens), Stuttgart, 8°.

Zeitschrift für analytische Chemie, Wiesbaden, 8°.

Zeitschrift für angewandte Chemie, Berlin, 4°.

Zeitschrift für anorganische Chemie, Hamburg und Leipzig, 8°.

Zeitschrift für öffentliche Chemie, Plauen i. V., 8° .

Zeitschrift für physikalische Chemie, Leipzig, 8°.

Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, Berlin, 8°.

Recueil des travaux chimiques des Pays-Bas et de la Belgique, Leide, 8°.

Gazzette chimica italiana, Roma, 8°.

Journal of the American Chemical Society, Easton, Pa., 8°.

Journal of the Chemical Society, London, 8°.

Berichte der deutschen chemischen Gesellschaft, Berlin, 8°.

Bulletin de la Société chimique de Paris, 8°.

Central-Blatt für Agrikulturchemie (Biedermanns), Leipzig, 8°.

Jahresbericht über die Fortschritte der Agriculturchemie, Berlin, 8°.

Chemische Industrie, Berlin, 4°.

Jahresbericht über die Leistungen der chemischen Technologie (Fischer), Leipzig, 8°.

Journal of the Society of Chemical Industry, London, 8°.

BIOLOGY.

Biochemisches Centralblatt, Leipzig, 8°.

Biological Bulletin of the Marine Biological Laboratory, Woods Holl, Mass., Lancaster, Pa., 8°.

Quarterly Journal of Microscopical Science, London, 8°.

Biologisches Centralblatt (Rosenthal), Leipzig, 8°.

Zeitschrift für Biologie, München und Berlin, 8°.

Archives de biologie (Beneden et Bambeke), Liége et Paris, 8°.

Archives italiennes de biologie, Turin, 8°.

Memoirs from the Biological Laboratory of the Johns Hopkins University, Baltimore, 8°.

Archives des sciences biologiques, St.-Pétersbourg, 8°.

Comptes rendus . . . de la Société de biologie, Paris, 8°.

BOTANY.

Botanical Gazette, Chicago, 8°.

Bulletin of the Torrey Botanical Club, Lancaster, Pa., 8°.

Contributions from the United States National Herbarium, Washington, 8°.

Journal of Mycology, Columbus, Ohio, 8°.

Missouri Botanical Garden Reports, St. Louis, 8°.

Annals of Botany (Balfour), London, 8°.

Annals of the Royal Botanic Garden, Calcutta, 4°.

Curtis's Botanical Magazine, London, 8°.

Gardeners' Chronicle. See Supplemental list, Bureau of Agriculture.

Hooker's Icones plantarum, London, $8^{\circ}.$

Index Kewensis plantarum phanerogamarum, 1. and 2. supplements, Brussels and Oxford, 4°.

Indian Forester. See Supplemental list, Bureau of Agriculture and Bureau of Forestry.

Journal of Botany, British and Foreign, London, 8°.

Journal of the Linnean Society, Botany, London, 8°.

Kew Bulletin of Miscellaneous Information, London, 8°.

Records of the Botanical Survey of India, Calcutta, 8° .

Transactions of the Linnean Society, 2. series, Botany, London, 4°.

Allgemeine botanische Zeitschrift, Karlsruhe, 8°.

Beihefte zum botanischen Centralblatt, Jena, 8°.

Berichte der deutschen botanischen Gesellschaft, Berlin, 8°.

Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie (Engler), Leipzig, 8°.

Botanische Zeitung, Leipzig, 4°.

Botanisches Centralblatt, Leiden, 8°.

Flora, oder Allgemeine botanische Zeitung, Marburg, 8°.

Genera siphonogamarum (De Dalla Torre et Harms), Leipzig, 4°.

Hedwigia. Organ für Kryptogamenkunde und Phytopathologie, Dresden, 8° .

Jahrbücher für wissenschaftliche Botanik (Pringsheim), Leipzig, 8.°

Just's Botanischer Jahresbericht, Leipzig, 8°.

Notizblatt des königl. botanischen Gartens und Museums zu Berlin, Leipzig, 8°.

Oesterreichische botanische Zeitschrift, Wien, 8°.

Pflanzenreich (Engler), Leipzig, 8°.

Zeitschrift für Pflanzenkrankheiten, Stuttgart, 8°.

Annales du Jardin botanique de Buitenzorg, Leide, 8°.

Annales des sciences naturelles, Botanique, Paris, 8°.

Bulletin de l'Herbier Boissier, Genève, 8°.

Journal de botanique, Paris, 8°.

Revue mycologique, Toulouse, 8°.

Malpighia, Genova, 8°.

Mededeelingen uit 'sLands Plantentuin, Batavia, 8.°

ZOOLOGY.

GENERAL.

Zoological Record, London, 8°.

Zeitschrift für wissenschaftliche Zoologie, Leipzig, 8°.

Zoologischer Anzeiger, Leipzig, 8°.

Archives de parasitologie (Blanchard), Paris, 8°.

Annotationes zoologicae Japonenses (Nihon Dobutsugaku Iho), Tokyo, 8°.

ENTOMOLOGY.

Canadian Entomologist, London, Ontario, 8°.

Entomological News, Philadelphia, 8°.

Entomologist, London, 8°.

Entomologist's Monthly Magazine, London, 8°.

Berliner entomologische Zeitschrift, Berlin, 8°.

Entomologische Zeitschrift, Guben, 4°.

Exotische Käfer (Heyne), Leipzig, 4°.

Exotische Schmetterlinge (Hübner), Leipzig, 4°.

Stettiner entomologische Zeitung, Stettin, 8°.

Wiener entomologische Zeitung, Wien, 8°.

Zeitschrift für Entomologie, Berlin, 8°.

Zeitschrift für Hymenopterologie und Dipterologie, Teschendorf i. Mecklenburg, 8°.

Genera insectorum (Wytsman), Bruxelles, 4°.

Revue d'entomologie, Caen, 8°.

Entomologisk Tidskrift, Stockholm, 8°.

Proceedings of the Entomological Society of Washington, 8°.

Transactions of the American Entomological Society, Philadelphia, 4° .

Transactions of the Entomological Society of London, 8°.

Annales de la Société entomologique de Belgique, Bruxelles, 8°.

Annales de la Société entomologique de France, Paris, 8°.

Bulletin de la Société entomologique, Paris, 8°.

ORNITHOLOGY.

Bulletin of the British Ornithologist's Club, London, 8°. Ibis, a Quarterly Journal of Ornithology, London, 8°.

MEDICINE.

INDEXES, BIBLIOGRAPHIES, REVIEWS, ETC.

Index Catalogue of the Library of the Surgeon-General's Office, United States Army, 2. series, Washington, 8°.

Index Medicus, 2. series, Washington, 8°.

Medical Review of Reviews, New York and London, 8°.

Medical Review, London, 8°.

Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, part 1, Referate, Jena, 8°.

Centralblatt für innere Medicin, Leipzig, 8°.

Centralblatt für die medicinischen Wissenschaften, Berlin, 8°.

Jahresbericht der Pharmacie, Göttingen, 8°.

Jahresbericht über die Fortschritte in der Lehre von den Gärungs-Organismen (Koch), Leipzig, 8°. Jahresbericht über die Fortschritte in der Lehre von den pathogenen Mikroorganismen (Baumgarten), Leipzig, 8°.

Jahresbericht über die Fortschritte der Physiologie (Hermann), Stuttgart, 8°.

Jahresbericht über die Fortschritte und Leistungen auf dem Gebiete der Hygiene (Uffelmann), Braunschweig, 8°.

Jahresbericht über die Leistungen und Fortschritte in der gesammten Medicin (Waldeyer und Posner), Berlin, 8°. Continuation of Virchow und Hirsch's Jahresbericht.

Schmidt's Jahrbücher der in- und ausländischen gesammten Medicin, Leipzig, 4°.

Bulletin de l'Institut Pasteur, Paris, 8°.

ANATOMY AND PHYSIOLOGY.

Anatomische Hefte (Merkel und Bonnet. Beiträge und Referate zur Anatomie und Entwicklungsgeschichte, part 1), Wiesbaden, 8°.

Anatomischer Anzeiger, Jena, 8°.

Archiv für Anatomie und Physiologie (Anatomische Abtheilung = Archiv für Anatomie und Entwicklungsgeschichte; Physiologische Abtheilung = Archiv für Physiologie), Leipzig, 8°.

Archiv für mikroskopische Anatomie und Entwicklungegeschichte, Leipzig, 8°.

Archives d'anatomie microscopique, Paris, 8°.

Journal de l'anatomie et de la physiologie normales et pathologiques, Paris, 8°.

American Journal of Physiology, Boston, 8°.

Journal de physiologie et pathologie générale, Paris, 8°.

Hoppe-Seyler's Zeitschrift für physiologische Chemie, Strassburg, 8°.

HYGIENE AND SANITATION.

Bulletins of the Hygienic Laboratory, Public Health and Marine-Hospital Service of the United States, Washington, 8°.

Public Health Reports (United States Public Health and Marine-Hospital Service), Washington, 8°.

Journal of Hygiene, Cambridge, 8°.

Arbeiten aus dem kaiserlichen Gesundheitsamte, Berlin, 8°.

Archiv für Hygiene, München und Berlin, 8°.

Archiv für Schiffs- und Tropen-Hygiene (Mense), Leipzig, 8°.

Hygienische Rundschau, Berlin, 8°.

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In the book lists following, the classification scheme is a tentative one based on experience in the use of the library in the past. As noted in the introduction, the books will be listed and placed on the shelves as rapidly as possible according to a slightly modified adaptation of the Dewey system of classification.

Several lists might well have been subdivided more closely, but the comparatively small number of books in such lists, the limited time available for the preparation of this catalogue, and the fact that the books are used to a large extent by the same people have led to their being placed under one alphabet.

Some books used extensively by workers in two divisions of scientific work—e. g., chemistry and medicine—have been included in both lists. This is true also of a few of the reference books which belong strictly to one branch of science but which are referred to so frequently that they have been placed on reference-book shelves in the library and included here under that division as well as in the subject list to which they belong.

The number of pages in books and pamphlets has been given only in the entomological works, where the large number of short monographs and reprints has made this seem advisable.

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In addition to the above periodicals, books to the number of 112 are still undelivered, distributed among the various divisions of the laboratory as follows: (1) Entomological works, consisting chiefly of articles published in scientific journals of which reprints have not as yet been secured, 52 volumes; (2) works on chemistry and chemical technology, 18 volumes; (3) botanical works, 29 volumes; (4) works in medicine and allied sciences, 12 volumes, and (5) photographic publications, 1 volume.

Some of these, particularly old editions, have not yet been found in the market; others are waiting for new editions to appear, and a small number are yet in manuscript, although announced when ordered.

SUPPLEMENTAL LIST OF BOOKS RECEIVED FROM VARIOUS BUREAUS OF THE DEPARTMENT OF THE INTERIOR.

The following supplemental lists include books transferred from various Bureaus of the Department of the Interior after the plan for the catalogue of the books previously in the library of the Bureau of Government Laboratories had been completed. They were added thus in supplemental lists (1) for lack of time in which to work out the classification scheme carefully for the whole list of books, and (2) because it was thought there was an advantage in showing what books were in the libraries of the various Bureaus before the transfer. It is believed that the table of contents and index are worked out sufficiently comprehensively to obviate especial difficulty from books being included in different lists.

In the case of some important botanical literature cross references are made from supplemental lists to lists of books in the library before transfer. This has not been carried further for lack of time.

Among the books of The Ethnological Survey were found many short monographs and reprints from scientific publications. For this reason the number of pages have been given for this list alone.

The use of the asterisk (*) before titles in the "Supplemental list" indicates that these books have been returned on memorandum receipt to the Bureaus from which they were received by transfer.

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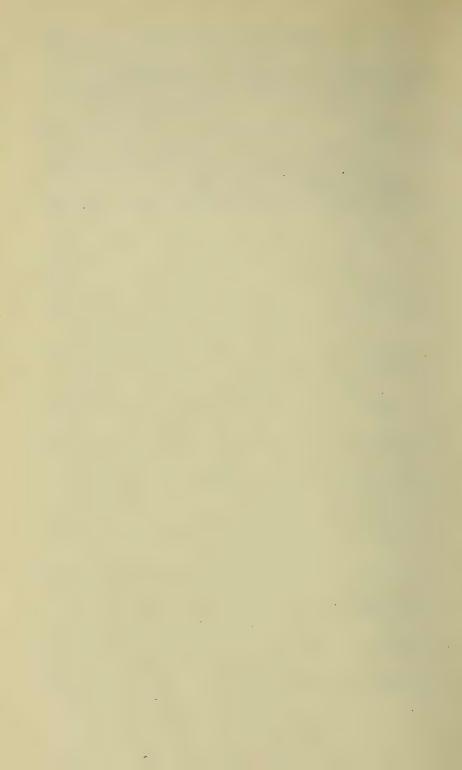
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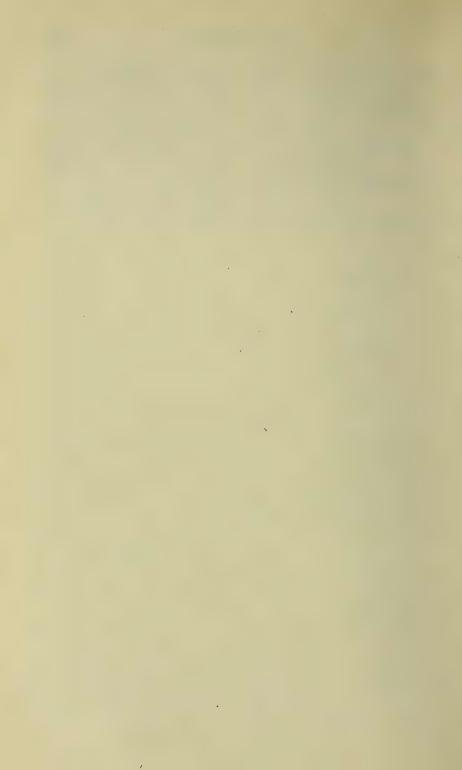
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 - VIII. Front stairway.
 - IX. Rear view showing serum laboratory power house and stack.
 - X. Babcock and Wilcox boilers.
 - XI. Corner of boiler room showing fire pump, filters, etc.
 - XII. Corner of the engine room.
 - XIII. Room in the chemical laboratory showing type of central desk.
 - XIV. Room in the biological laboratory showing type of central desk.
 - XV. One of the larger hoods in the chemical laboratory.
 - XVI. Type of room in chemical laboratory.
 - XVII. Main room devoted to physics and physical chemistry.
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 - XXII. Library (central room).
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- XXIV. Library (east alcove).
- XXV. Showing metal stack in library.
- XXVI. Library, showing one of reading tables.
- XXVII. Floor plan of library rooms.

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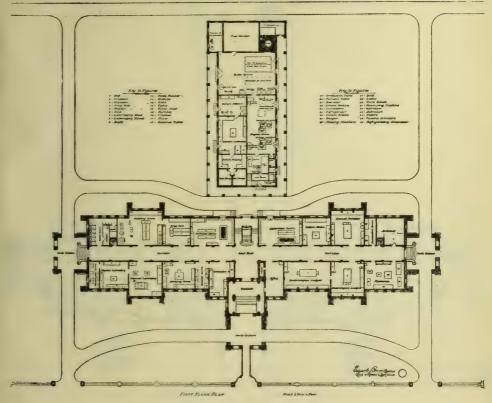
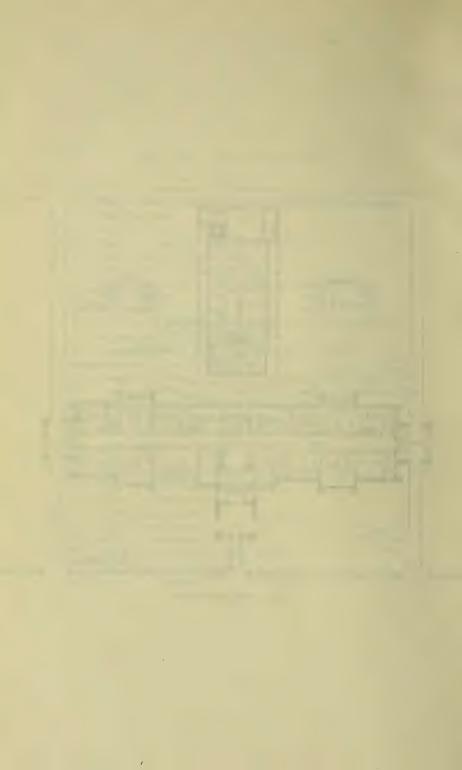


PLATE I. FIRST FLOOR PLAN.



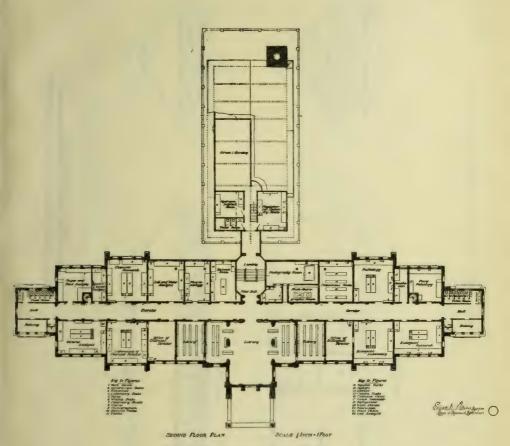
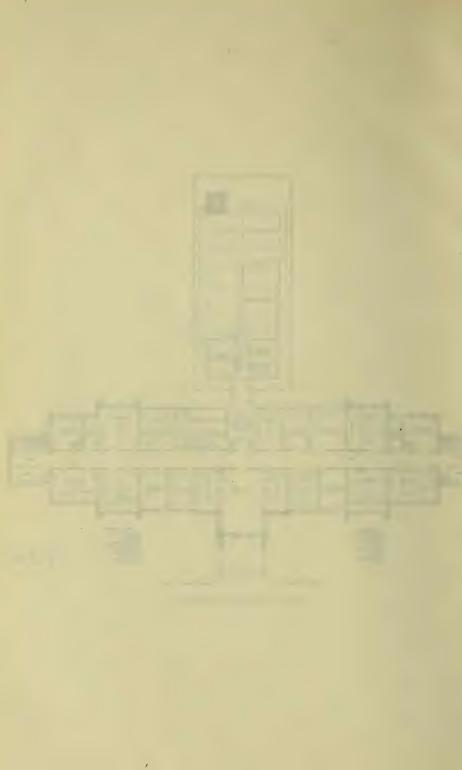
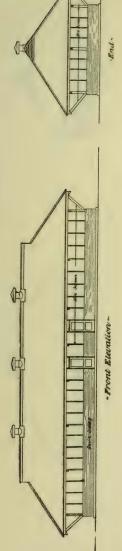
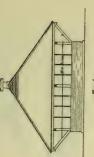


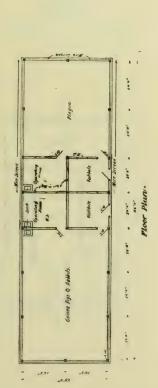
PLATE II. SECOND FLOOR PLAN.

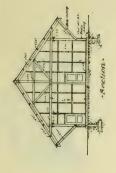


Animal Houses Bureau of Gov: Laworatories Guinea Pigs, Rabbils & Plague Animals.











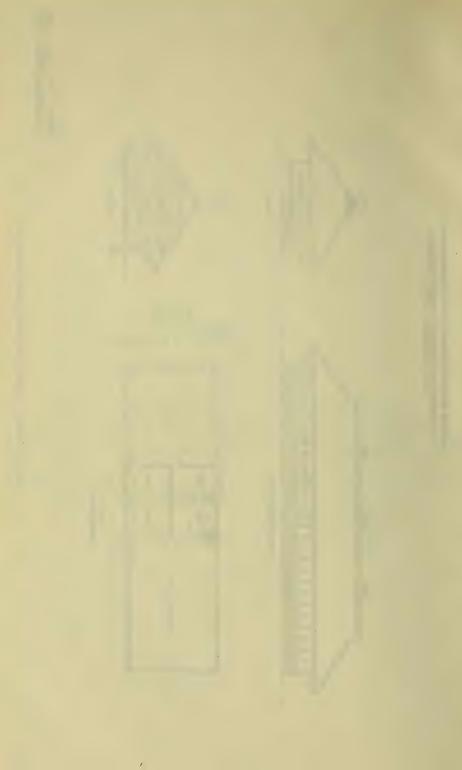


PLATE IV. PLANS OF STABLE FOR SERUM HORSES.



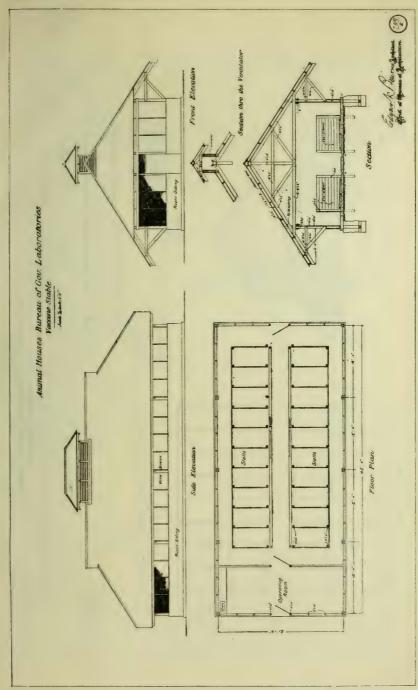


PLATE V. PLANS OF STABLE FOR VACCINE CALVES.



PLATE VI. PLANS OF HOUSES FOR DOGS, GOATS, SHEEP, AND MONKEYS.

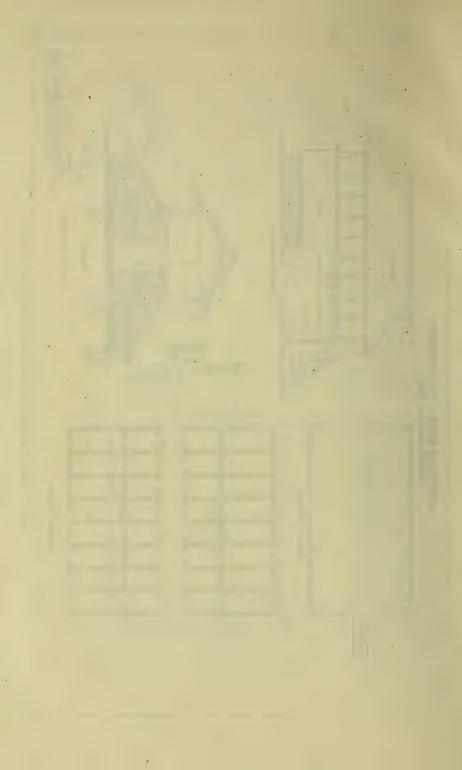


PLATE VII. MAIN ENTRANCE.

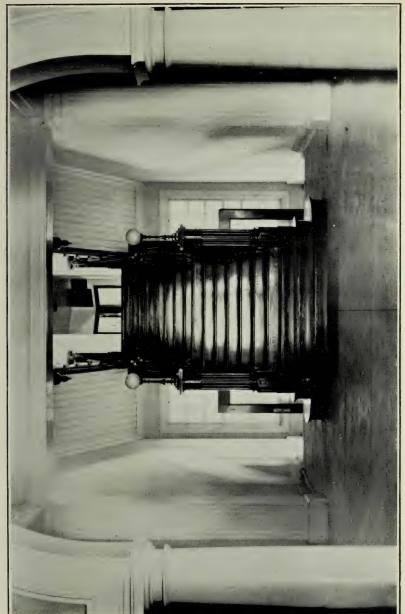


PLATE VIII. FRONT STAIRWAY.

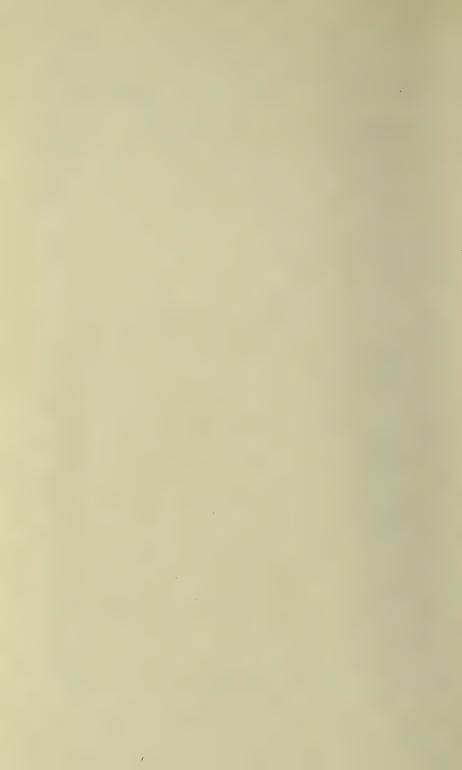
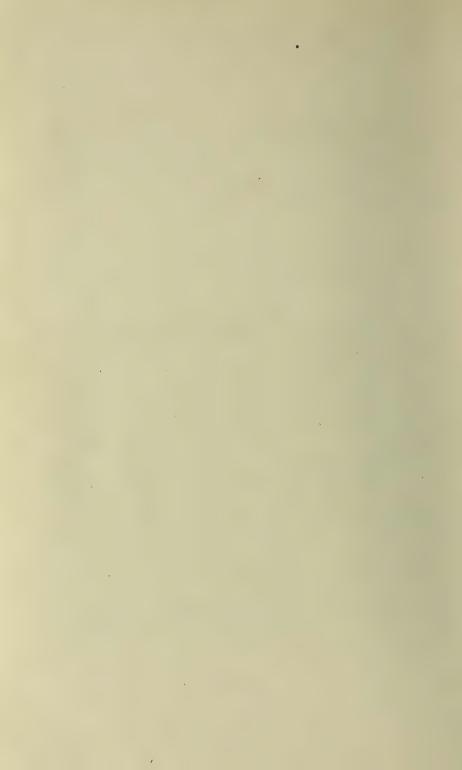




PLATE IX. REAR VIEW SHOWING SERUM LABORATORY POWER HOUSE AND STACK.



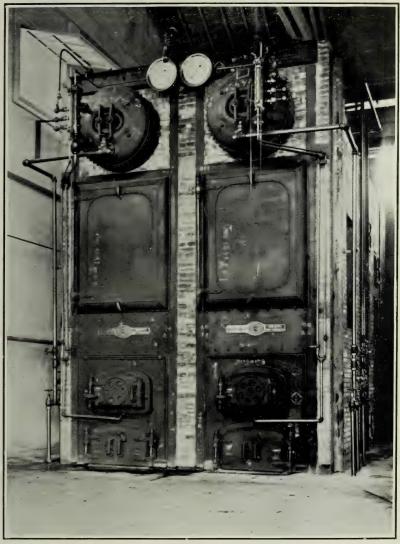


PLATE X. BABCOCK AND WILCOX BOILERS.

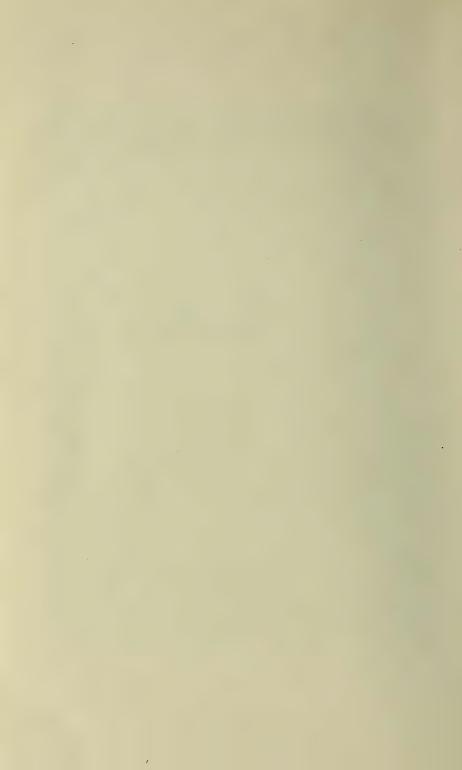
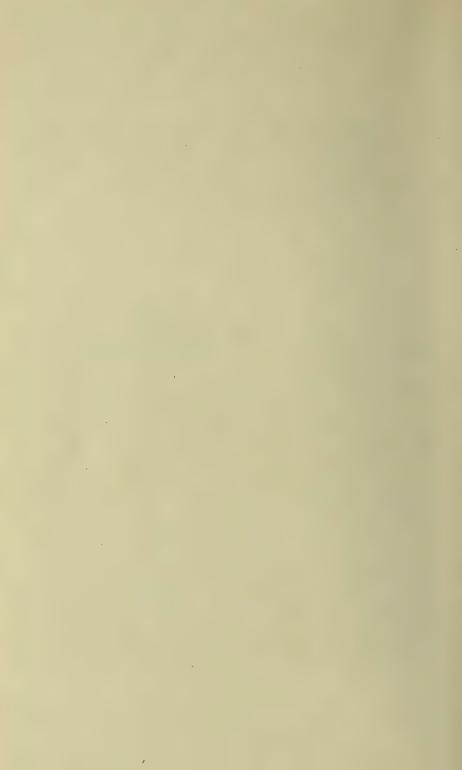




PLATE XI. CORNER OF BOILER ROOM SHOWING FIRE PUMP, FILTERS, ETC.



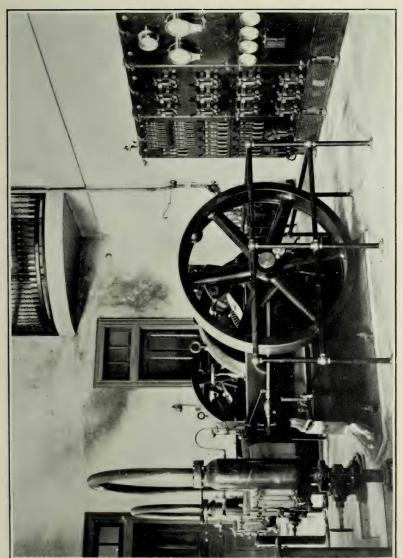


PLATE XII. CORNER OF THE ENGINE ROOM.

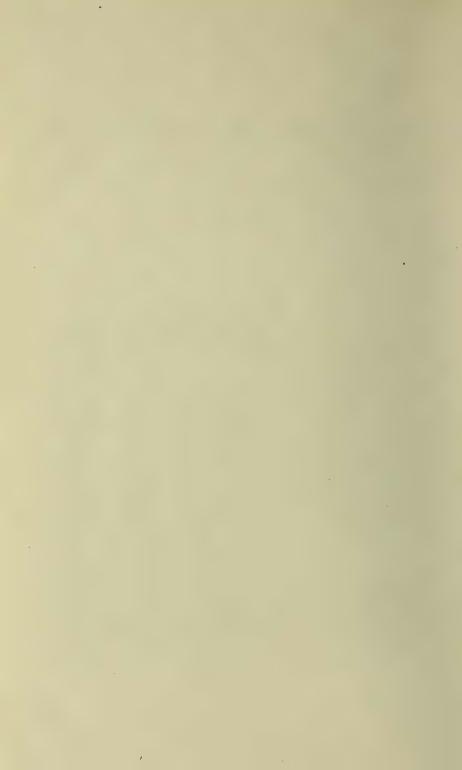




PLATE XIII. ROOM IN THE CHEMICAL LABORATORY SHOWING TYPE OF CENTRAL DESK.

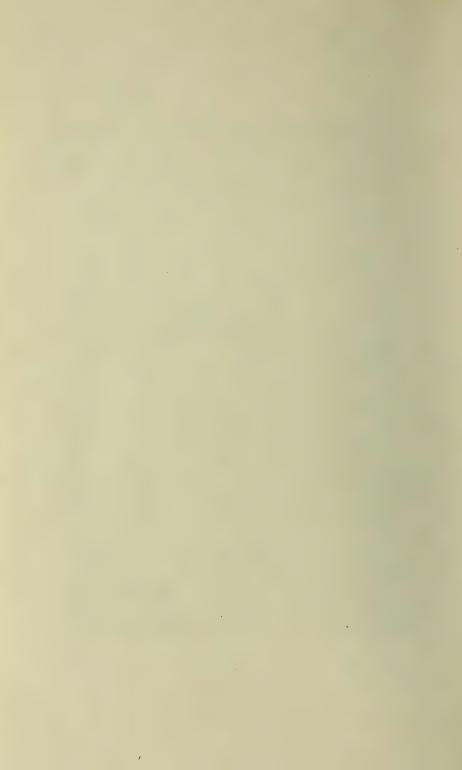
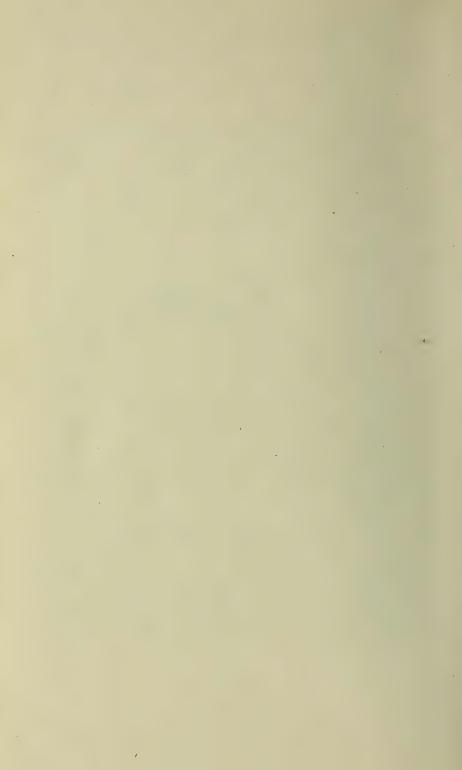




PLATE XIV. ROOM IN THE BIOLOGICAL LABORATORY SHOWING TYPE OF CENTRAL DESK.



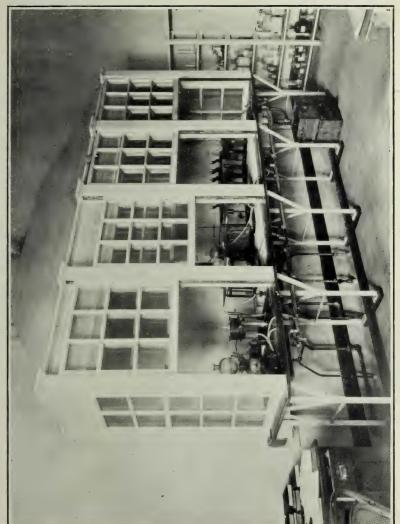


PLATE XV. ONE OF THE LARGER HOODS IN THE CHEMICAL LABORATORY.

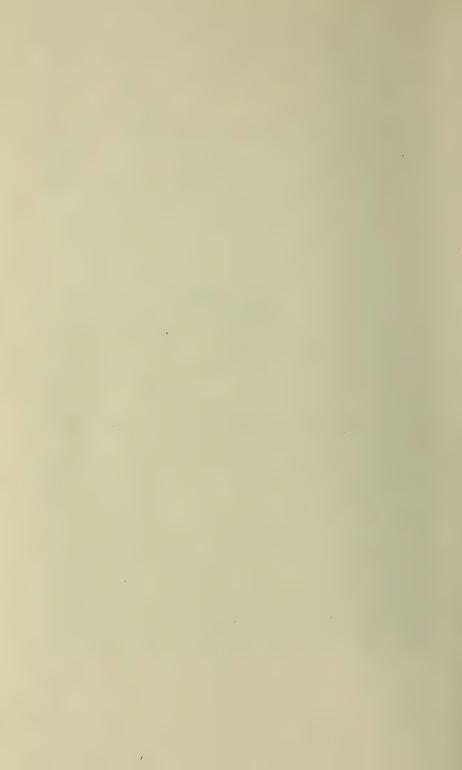
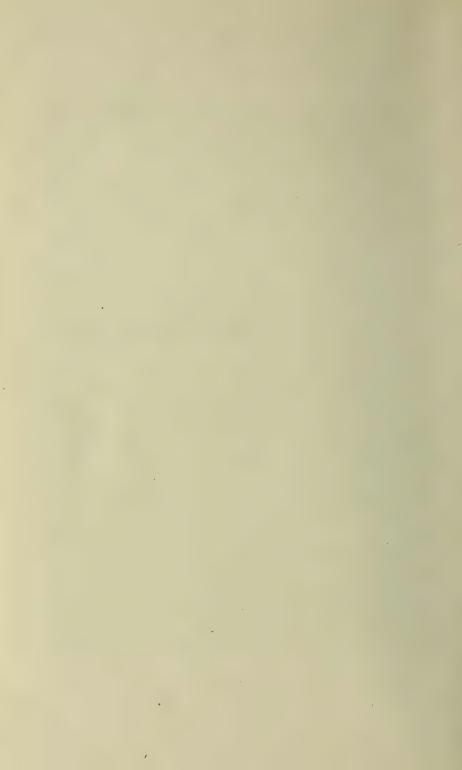




PLATE XVI. TYPE OF ROOM IN CHEMICAL LABORATORY.



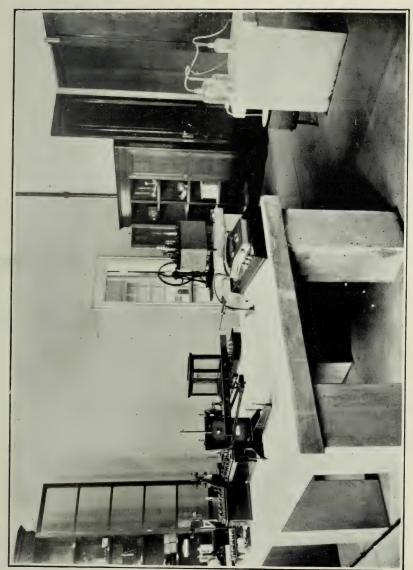
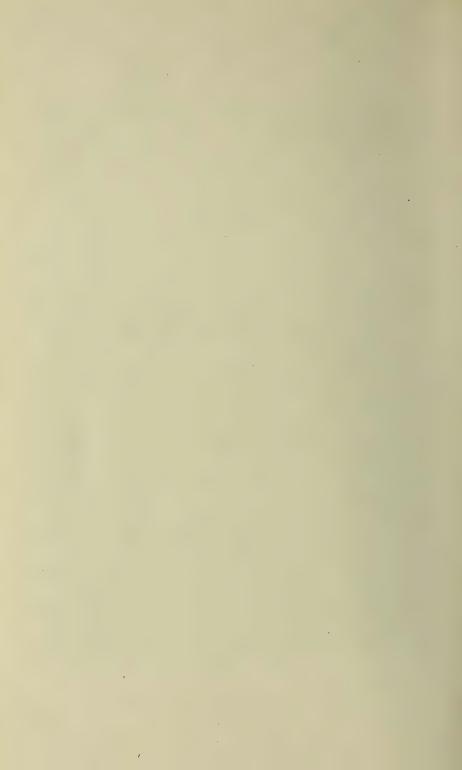


PLATE XVII. MAIN ROOM DEVOTED TO PHYSICS AND PHYSICAL CHEMISTRY.



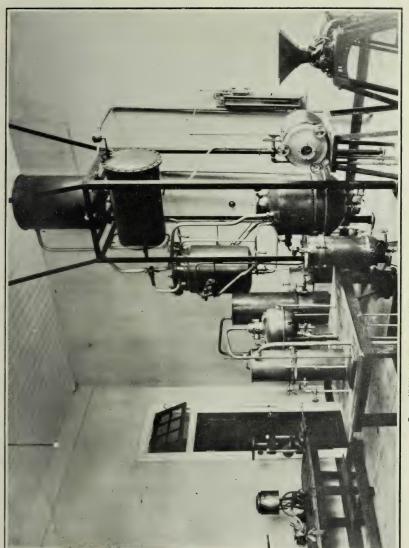


PLATE XVIII. VACUUM DRYING AND DISTILLING APPARATUS, ETC.

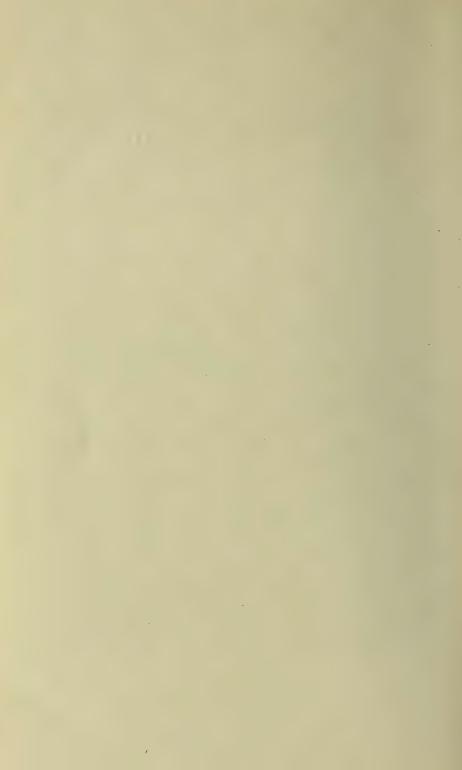


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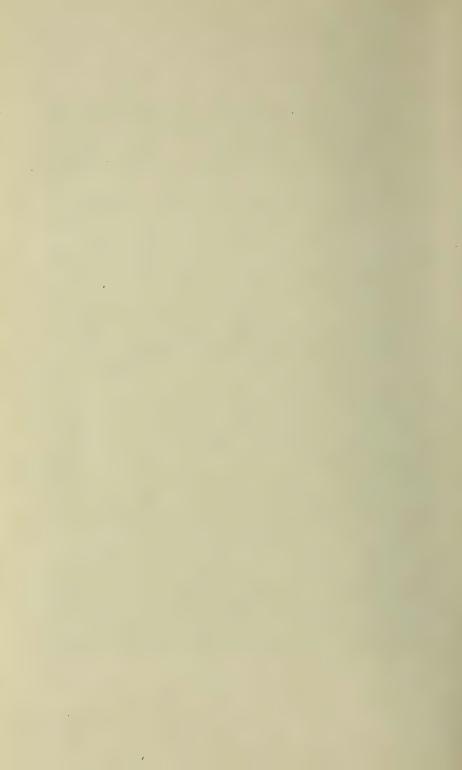
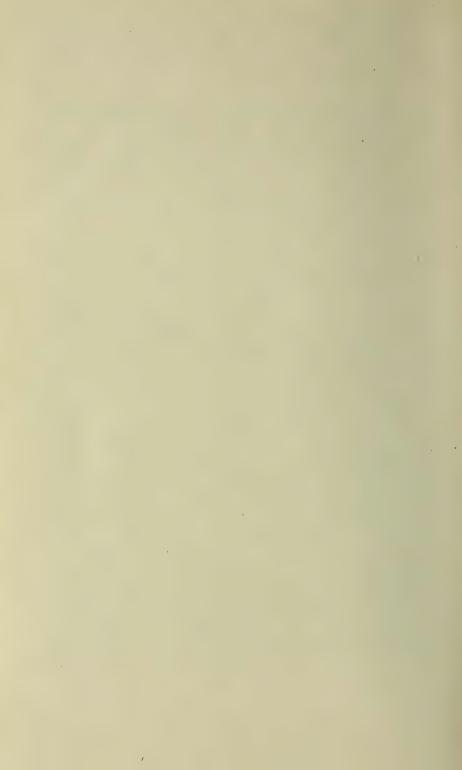




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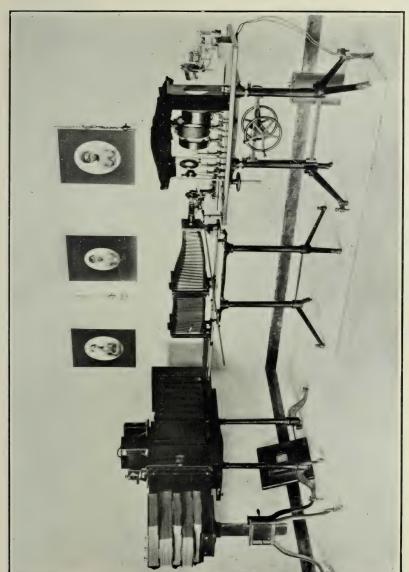


PLATE XXI. CORNER OF PHOTOGRAPHER'S STUDIO.

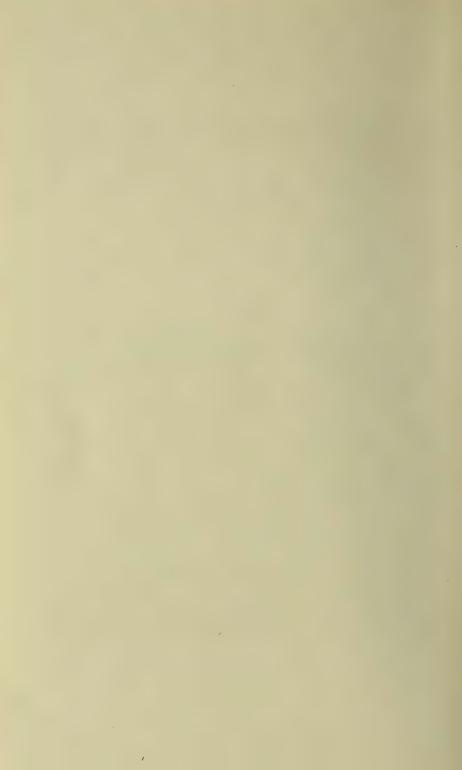




PLATE XXII. LIBRARY (CENTRAL ROOM).

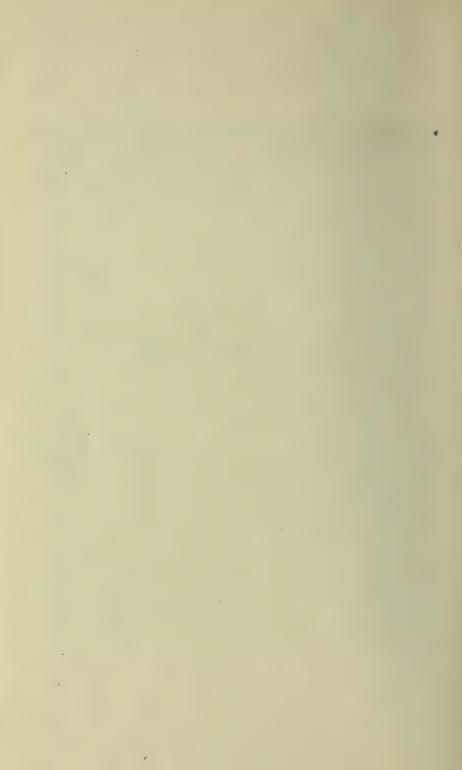




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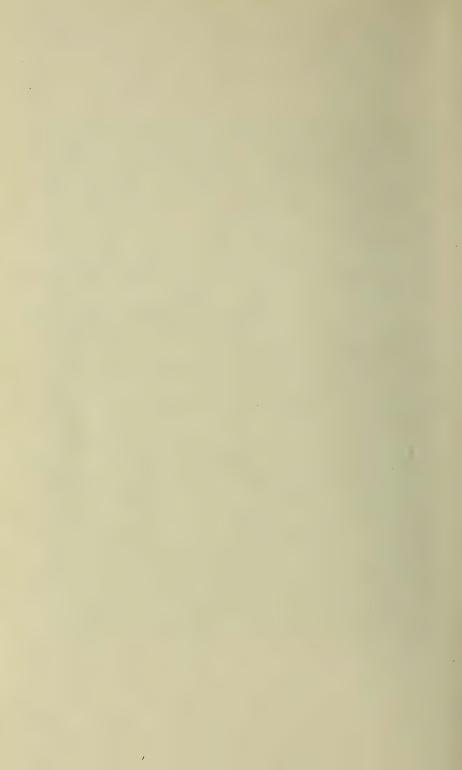




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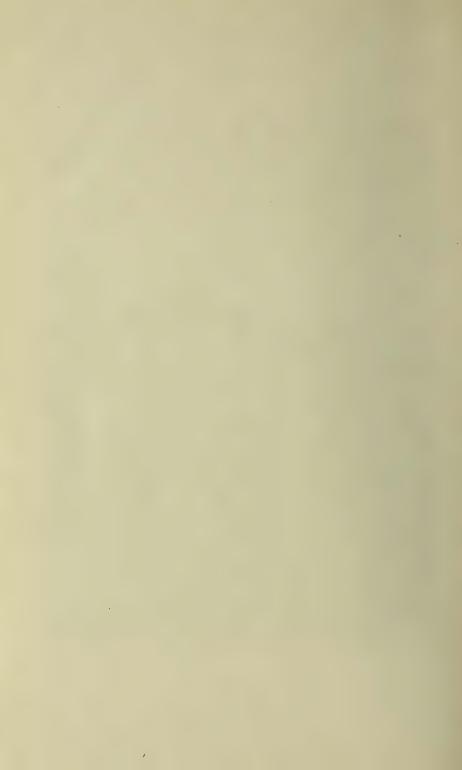




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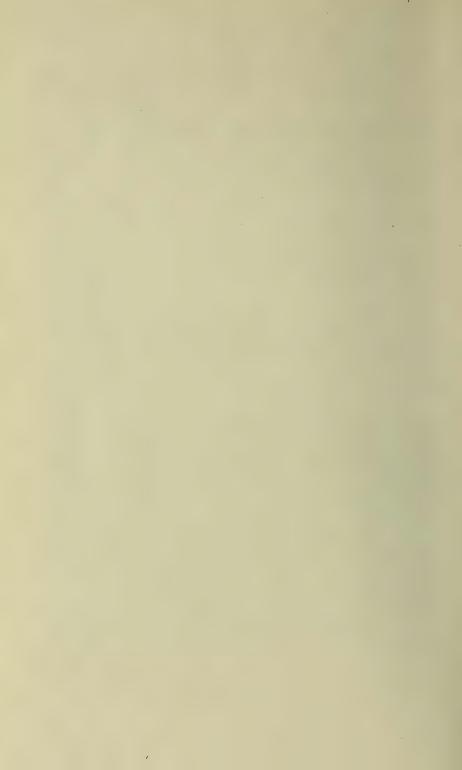


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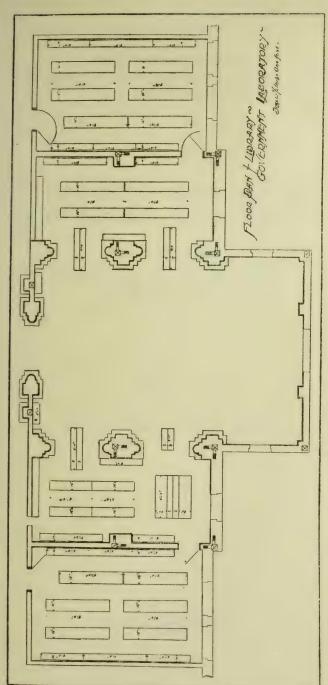
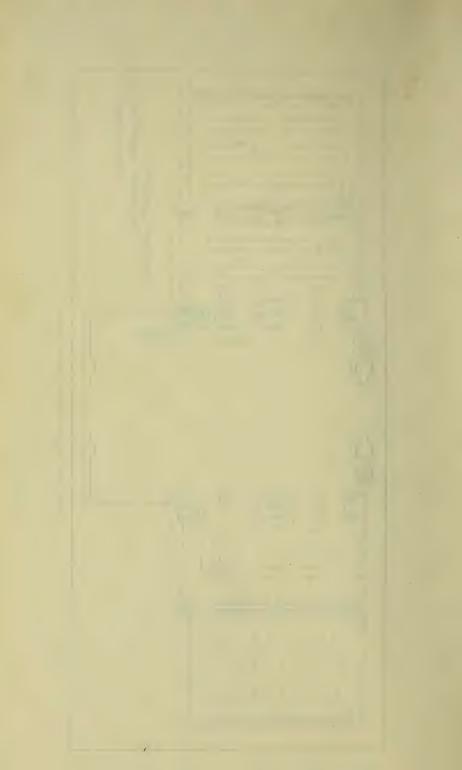


PLATE XXVII. FLOOR PLAN OF LIBRARY ROOMS.



No. 23.-OCTOBER, 1904

DEPARTMENT OF THE INTERIOR BUREAU OF GOVERNMENT LABORATORIES BIOLOGICAL LABORATORY

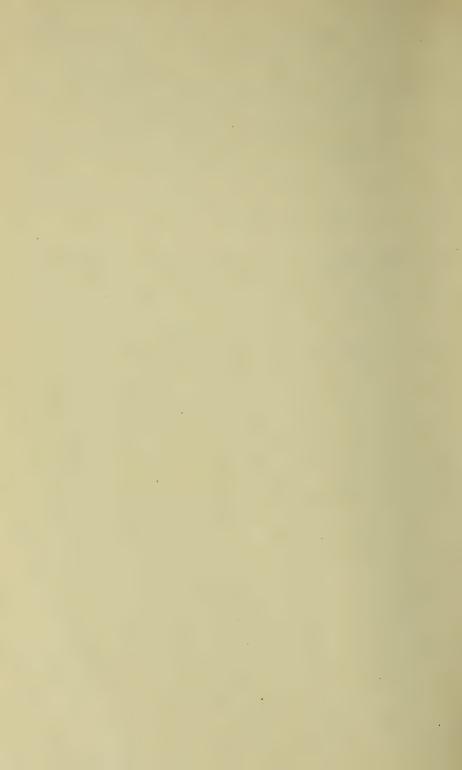
THE PLAGUE: BACTERIOLOGY, MORBID ANATOMY, AND HISTOPATHOLOGY

INCLUDING A CONSIDERATION OF INSECTS
AS PLAGUE CARRIERS

BY

MAXIMILIAN HERZOG, M. D.

MANILA
BUREAU OF PUBLIC PRINTING
1904



LETTERS OF TRANSMITTAL.

Department of the Interior,
Bureau of Government Laboratories,
Office of the Superintendent of Laboratories,
Manila, October 20, 1904.

SIR: I have the honor to transmit herewith an article entitled "The Plague: Bacteriology, Morbid Anatomy, and Histopathology (including a consideration of insects as plague carriers)," by Maximilian Herzog, M. D.

I am, very respectfully,

PAUL C. FREER,

Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER,

Secretary of the Interior, Manila, P. I.

Department of the Interior,
Bureau of Government Laboratories,
Biological Laboratory, Office of Director,
Manila, October 19, 1904.

SIR: I have the honor to transmit herewith and to recommend for publication a report entitled "The Plague: Bacteriology, Morbid Anatomy, and Histopathology (including a consideration of insects as plague carriers)," by Dr. Maximilian Herzog, pathologist Biological Laboratory.

Very respectfully,

RICHARD P. STRONG, Director Biological Laboratory.

Dr. PAUL C. FREER,

Superintendent Government Laboratories.

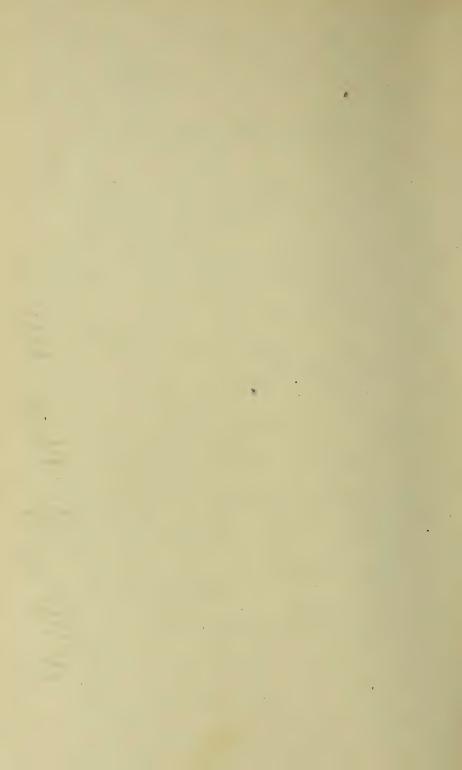
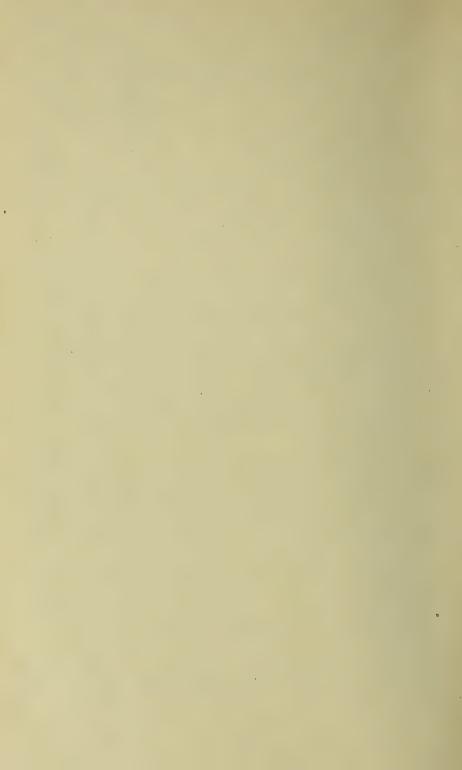


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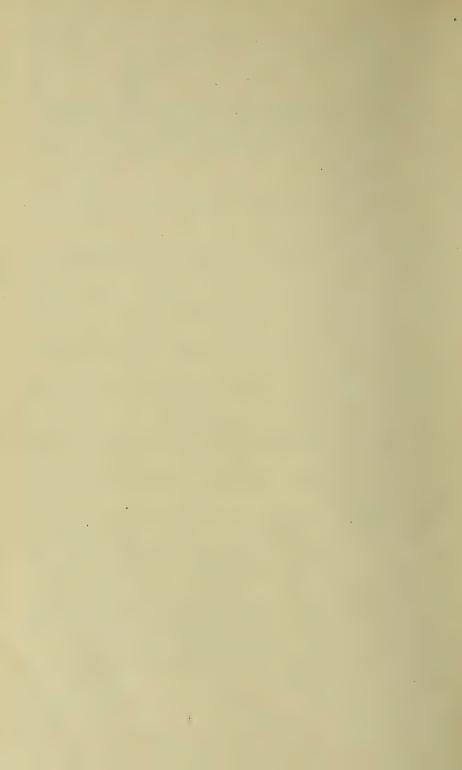


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THE PLAGUE: BACTERIOLOGY, MORBID ANATOMY, AND HISTOPATHOLOGY.

(INCLUDING A CONSIDERATION OF INSECTS AS PLAGUE CARRIERS.)

By Maximilian Herzog, M. D., Pathologist Biological Laboratory.

PREFACE.

The "Great Black Death," the most dreaded scourge of the Middle Ages—the grim reaper who, as is estimated, in the four-teenth century, within three years, carried away in Europe twenty-five millions of people—had, as it seemed, vanished from the surface of the globe, when about ten years ago a new great pandemic of plague, which still persists, appeared in China and India. From here it made excursions into London, Oporto, Glasgow, Triest, Alexandria, Sidney, Hamburg, Bremen, and Naples—however (thanks to the modern methods of meeting infectious and contagious diseases), without there gaining a foothold or assuming dangerous epidemic proportions.

At present the dreaded disease has not merely reached our own Far Eastern, transoceanic possessions—the Philippine Islands—but it has even established itself endemically in California at San Francisco. While it is to be hoped that with proper vigilance and with a continuous, untiring practice of the prophylactic measures which we are taught by modern hygiene the plague may never assume dangerous epidemic proportions, either in the Philippines or in the United States, we have every reason to keep it in view and to study it carefully in all of its phases. Therefore we need not apologize for offering in this bulletin a contribution to the bacteriology, morbid anatomy, and histopathology of plague as gained from both a perusal of literature and more particularly from

a study of a number of cases which came to post-mortem examination in Manila.

It has been my purpose in the preparation of this report to furnish a scientific contribution to the subject and to bring out, if possible, some new points, which might be of interest and value. However, another practical object has never been lost sight of, namely, to provide a guide for those who might be called upon to make a post-mortem diagnosis of the first case of suspected plague in a district. This will always be a matter of great responsibility and sometimes one of considerable difficulty. Unless circumstances compel the contrary, the diagnosis of a plague case, being the first of its kind in a community, on board of a ship, etc., should be confirmed by one well trained in general pathology and bacteriology; or, still better, by one also having experience in the recognition and examination of plague cases.

The importance of promptly diagnosing the first case of plague can not be overestimated. This disease does not make its initial appearance as a widespread epidemic, as is often the case with Asiatic cholera, when caused by a contaminated water supply or from other sources of infection. Rather, it begins very insidiously from one or several imported cases. It is frequently easy to hold the plague in check, provided it is recognized and properly fought at an early stage. This has been proved by the observations made in the cities mentioned above and in Manila.

In studying the histopathology of plague a highly interesting change was found in the kidneys, namely, extensive and frequently occurring hyaline fibrin thrombosis of the glomerular capillaries. As it appears that this change has not been described in the study of the microscopic anatomy of the disease, it has been considered somewhat at length. The histologic examinations in their entirety have led to the conviction that plague in its most common bubonic type should not be looked upon as a hemorrhagic septicæmia, because according to all appearances the infecting bacilli remain practically localized until the agonal stage of the disease is reached.

One of the cases examined and herein reported in detail suggested the possibility, if not the probability, of an infection through the agency of pediculi. This led to the study of the rôle which insects may play as carriers of plague. The ones which have been accused particularly of being the most important factors in

spreading plague from rats to men are the rat fleas. In taking up this question with reference to local conditions, it was found that these rodents in Manila are infested by a species of *siphonaptera* not heretofore described. It, however, appears that this new species of flea has not taken any part in the conveyance of the disease, which may or may not have been first imported to this city through the agency of rats.

It is a source of great satisfaction to the writer to be in a position to present this paper well illustrated with colored plates, photographs, and microphotographs, by which whatever value it possesses, both as a scientific contribution and as a guide in the postmortem diagnosis of plague, is undoubtedly greatly enhanced.

For a revision of the manuscript the author is indebted to Drs. Paul C. Freer and Richard P. Strong. The colored drawings were prepared by Mr. T. Espinosa and Mr. W. Schultze and most of the microphotographs by Mr. Chas. Martin, all members of our laboratory staff.

THE DISCOVERY OF THE PLAGUE BACILLUS.

The etiologic factor of plague in all of its various forms is a specific micro-organism—the plague bacillus. It can generally, without difficulty, be cultivated in pure culture from cases of plague in man. In susceptible animals it produces a disease identical with or similar to that found in the human race, and in some deplorable laboratory accidents, in locations where no plague existed, it has caused typical fatal pest infection in men. The names both of Yersin and of Kitasato are connected with the discovery of the plague bacillus during the epidemic in Hongkong in 1894. To the claims of each of these investigators to be the independent discoverer of the plague germ one may well apply the words of the German poet, that it is "Von den Parteien Hass und Gunst entstellt."

Some writers upon plague dispute Yersin's claim; while not a few, and particularly his own countrymen, maintain that Kitasato is not entitled to any fame in connection with the original finding of *Bacillus pestis*. Ogata, for instance, tersely states:

Many authors believe that the plague bacillus of Kitasato and that of Yersin are identical, but such is not the case. They are two absolutely different kinds of bacilli.

And then Ogata as well as Yamagiwa, in another publication, shows that they found the Yersin bacillus as the cause of the plague in the Formosa epidemic. Scheube equally opposes Kitasato's claim and says:

The bacillus discovered simultaneously and at the same place by Kitasato is not the true plague bacillus (discovered by Yersin), because it shows marked points of difference, it is motile, etc.

It is a somewhat ludicrous misfortune which befalls Scheube in his polemical remarks against Kitasato, that he himself confuses some of the differentiating characters of Yersin's and Kitasato's first descriptions. Scheube makes it appear as if Kitasato at first erroneously stated that the plague bacillus does not and that Yersin's bacillus does retain Gram's stain. The opposite, of course, is true. Yersin correctly, in his first report, stated that the plague bacillus loses Gram's stain.

On the other hand, Cantlie, of the Indian Medical Service, at a plague conference and discussion held in London in 1898 observed that the names of Kitasato and Yersin were usually associated, and the bacillus was commonly termed the Kitasato-Yersin bacillus. Kitasato, however, had demonstrated the bacillus a week previous to Yersin, and although he (Cantlie) felt the greatest respect for the work of Yersin on plague, yet the latter had no claim as an independent discoverer of the bacillus, since that distinction belonged to Kitasato alone. (Brit. Med. Jour., 1898, Vol. II, Sept. 24, p. 962.)

However, anyone who reads the reports of Yamagiwa and Ogata regarding the epidemic in Formosa, which contain literal translations from Kitasato's first publications in Japanese, can not fail to conclude that it was indeed Yersin and not Kitasato who had first worked with pure cultures of the plague bacillus. Kitasato's first reports show clearly that what he described as pure cultures of the plague bacillus were not such. On the other hand, there can be no reasonable doubt that Kitasato saw and recognized plague bacilli shortly before Yersin. No bacteriologist examining the juice of buboes in certain stages, either from the living or from the post-mortem table, can overlook the enormous number of characteristic bacilli. However, it was undoubtedly Yersin who first isolated and described what was really a pure culture of the plague bacillus.

Yersin's first published description was submitted to the French Academy and reads as follows:

The first bacteriologic researches were made on living subjects. An examination of the blood drawn from the finger at various periods of the disease failed to show the microbes, and the inoculations remained sterile. The buboes, on the contrary, contained an abundance of a pure culture of a very small, short bacillus, with rounded ends, which does not stain by Gram's method, but does with Gentian violet. I have found this bacillus in buboes of eight patients, and in two plague autopsies I have found the same microbe. It is particularly numerous in the buboes, less abundant in the other (lymphatic) ganglions, and very rare in the blood at the moment of death. The liver and spleen are increased in size and contain the specific bacillus.

A short account of some animal experiments with post-mortem findings forms the conclusion of Yersin's first report. The second one gives an equally correct—in some respects more elaborate—description of the bacillus, of its cultivation, and of animal experiments. Kitasato, in his first description in the London Lancet (August 25, 1894), states that the bacilli are to be found in the blood, in the bubbes, in the spleen, and in all the internal organs of the victims of the disease.

I am at present [he says] unable to say whether or not Gram's doublestaining method can be employed. The bacilli show very little movement.

Metschnikoff's standpoint in the controversy as to the discovery of the plague bacillus is perhaps the most correct and the most impartial one, hence we quote his words in the language of the original. He says:

Yersin apres des recherches laborieuses effectuées dans des conditions particuliérement difficiles, decouvrit le microbe pesteux. Independamment de lui Kitasato arrivait au meme resultat. Le savant japonais s'est borné a communiquer quelques notes preliminaires sur ce sujet, tandis que Yersin en a poursouvi l'etude avec persévérance; c'est donc a lui que nous devons le meilleur de nos connaissances actuelles sur la peste.

MORPHOLOGY OF THE PLAGUE BACILLUS.

The bacillus pestis is quite variable in its morphology, and it is important to remember this fact in connection with the bacteriologic diagnosis of the disease. In post-mortem smears prepared from a recent nonsuppurating primary bubo, from a pneumonic focus, from the spleen, and occasionally from other internal organs one generally finds numerous plague bacilli. In those from the

primary bubo and from the pneumonic foci their number is legion. In smears from the spleen they are fairly abundant, while in preparations from the other organs, according to our own experience, they are generally present only to a moderate extent, unless septico-pyemic metastatic foci should have been established, when they may be quite numerous. In smears prepared from the heart's blood we have always found very few bacilli, even in pneumonic and septico-pyemic cases.

As a routine method for the examination of smears we have adopted the following procedure: The preparations were made during the post-mortem examinations on slides, and these, as soon as air-dry, were placed in a wide-mouthed bottle containing absolute alcohol. Here they remained until after the termination of the autopsy, when they were stained with dilute carbol-fuchsin (1 part of the original stain to 5 to 10 aqua destillata) for twenty to forty seconds, and then freely washed with water. They were next immediately examined with oil-immersion magnification. If it was found that the stain was not satisfactory, being either too light or too heavy, the immersion oil was washed off in xylol and the shortcomings corrected either by a second prolonged dyeing or by rapid immersion in alcohol, followed by washing in water. ever, after a little practice it is soon possible correctly to estimate the time necessary to bring about that particular stain which will best show the characteristic morphologic features of the bacillus, and a second attempt is rarely necessary. If after the first examination of the slides it is desirable to preserve them for future reference, the immersion oil is washed off in xylol and the specimens may now be mounted permanently in Canada balsam, being protected by a cover slip.

In smears made from the organs the plague organism appears as a rather short, plump bacillus, being 1.5 to 1.75 μ long and 0.5 to 0.75 μ thick; generally the proportion of width to length is as one to two. Individuals considerably longer than 1.75 μ are occasionally seen. The bacilli are generally single, occasionally diplobacilli are encountered, and very rarely short chains. In smears which have been fixed in absolute alcohol and which have been properly dyed the bacilli are not uniformly colored, but they show a distinct polar staining. Frequently the whole periphery of the bacillus is stained and only the center has remained uncolored. Other forms of the organism, while differing in certain respects

from the above-given description and while not representing the most characteristic type, are so frequently found in smears that the student of plague must thoroughly familiarize himself with them. These are elliptical or egg-shaped, or almost spherical, and they show only a very narrow peripheral staining, or they may not stain at all; hence they look like mere empty shells, which indeed they probably are, since they are most commonly found in older buboes. Also, in smears from cases which have succumbed only after several days' sickness, one frequently sees various involution forms of the plague micro-organism, which appear like yeast cells or which are either quite hazy and indistinct or club shaped and irregular in outline. Sata has shown that such involution forms may be seen on and after the fourth day in experimental plague infection in animals. In cover-glass preparations from pure cultures the bacilli are not so characteristic as we find them in smears from human plague cases, except in some involution forms to be more fully described below. The first generation, as a rule, shows the polar staining and the unstained center fairly well, but in subsequent ones these features often become more or less indistinct, or may even entirely be lost. Plague bacilli from pure cultures, particularly from the water of condensation of agar tubes, or from bouillon, frequently show shorter or longer chains, in which dividing lines between the individual bacilli are so indistinct as to cause them to appear like filaments. Involution forms are likewise liable to present themselves early even on favorable media. We have occasionally seen them as soon as the second or third day on agar tubes.

The plague bacillus may be stained with any one of the watery solutions of the common basic aniline dyes. The carbol-thionin stain, frequently mentioned in connection with it, is not to be recommended if one works in the Tropics, since for certain reasons it is very liable to fade. Kossel's modification of Romanowsky's stain has not been very satisfactory in our hands, and for general practical purposes we have found dilute carbol-fuchsin used in the manner above recommended the most simple and most uniformly reliable.

The plague bacillus, if treated by Gram's method, is decolorized. The bacillus, if grown in the animal body, possesses a capsule, which, however, is not very easy to demonstrate unless thin spreads are prepared and fixed in alcohol with great care. There is nothing

chracteristic in the capsule, so its exhibition will not assist in the microscopic diagnosis of plague. The bacillus is not motile, does not possess any flagellæ, nor has spore formation been observed. Even if one occasionally sees in the bacilli bodies somewhat like spores, they are not genuine spores, because such bacilli are not more resistant to heat, antiseptics, etc., than the other pest bacilli.

CULTIVATION OF THE BACILLUS.

The plague bacillus grows on all ordinary laboratory culture media, best on such as are faintly alkaline. Even a minor degree of acidity as well as a higher degree of alkalinity prevents its development. It develops at temperatures ranging from 5° to 38° C., and in our artificial media best at 25° to 30° C. It is quite strictly, though perhaps not absolutely, obligate aërobic. As a rule it develops on artificial media only in the presence of free oxygen. However, some observers have occasionally seen a weak growth even in the absence of the latter. When a favorable solid culture medium (agar or gelatin, slightly alkaline) is inoculated from the organs (bubo, spleen, etc.) of a plague case, the development of the plague bacilli is at first generally quite slow, and frequently very little can be seen with the naked eye within the first twenty-four hours. On the other hand, in a considerable number of cases a typical picture may appear after this time and it is always present after forty-eight hours. The surface of the agar or gelatin shows small, delicate, round, moist, dewdrop-like colonies. They are light gray in reflected light and gravish-white in transmitted. If these colonies are inspected with a hand lens or with a lower power of the compound microscope they show an elevated, finely granular, rounded center and a perfectly transparent, very thin, flat marginal zone. The colonies on the whole are circular, but the transparent marginal zone early shows a somewhat irregular boundary line. Colonies of this type are not formed by any other micro-organism, except occasionally by the influenza bacillus. However, in the case of the plague bacillus they develop most characteristically on ordinary gelatin plates kept at 20° to 24° C., under which conditions the influenza bacillus would not show any growth at all. While these typical young colonies are seen both on agar and on gelatin, we think that they are best shown on the latter, hence the use of this medium is to be greatly recommended in the bacteriologic diagnosis of plague. Gelatin plates can, of course, be

used in the Tropics only where an incubator with cooling device is at hand. If an impression preparation is made of one of the young colonies with a homogeneous, transparent, marginal zone, it is seen that this consists of bacilli arranged in curved filaments. If the inoculation from the organs has been quite rich in plague bacilli the surface between the colonies has a ground-glass appearance, which becomes more marked after forty-eight hours, and is generally well seen in cultures a few days old. After three or more days the erstwhile very delicate colonies become larger, more granular, and less transparent. The marginal zone likewise has become thickened, is less homogeneous, and its edges become more irregular and are often finely serrated at the periphery. The colonies now have a more decidedly grayish-white appearance and occasionally show a slight tinge of yellow. In young cultures of the first generation colonies are usually small, grow slowly and remain comparatively small, occasionally, however, rapidly attaining a diameter of several millimeters. We have several times seen such large colonies where the material from which an inoculation was made contained few bacilli and where subsequently only a few colonies developed. However, this is probably not the only condition under which the large ones make their appearance. Older cultures of the plague bacillus, particularly when they have in consequence of evaporation become dry and quite granular, are somewhat iridescent. If a young plague culture is touched with a platinum loop it is found to be viscous and sticky. It is, however, easily removed from the surface on which it grows.

The plague bacillus, as stated before, early in its growth has a marked tendency to develop involution forms. As first shown by Hankin, this tendency is most pronounced in cultures on a 3 to 4 per cent salt agar. Hence we have in this medium one of the most valuable means for the bacteriologic diagnosis of plague. It is prepared and standardized like any ordinary agar, only that it does not merely contain one-half but 3 to 4 per cent of common salt. Hence, in case of an emergency, salt agar can be prepared from the ordinary media by the addition of the proper amount of NaCl to bring it up to the desired concentration. We have found it best to use a 3.5 to 4 per cent salt agar and not to diminish this percentage. On a medium of this concentration, particularly if it is fairly dry and contains very little water of condensation, involu-

tion forms of the plague bacillus are seen after twenty-four hours Generally the greater number, or all, of the organisms from such a growth present themselves as large spherical bodies, looking very much like yeast cells; later, large swollen club or dumbbell shaped, spermatozoa-like or irregular forms make their appearance. most typical, and the most constant form on a 3.5 to 4 per cent salt agar, after twenty-four to forty-eight hours, is the yeast-like, large, spherical plague organism. There is no micro-organism which forms this type so promptly and so regularly on salt agar and which might be confounded with the plague bacillus. Hence it is advisable in a first suspected plague case at the autopsy to inoculate besides gelatin plates also ordinary agar tubes, bouillon flasks, and salt agar tubes or plates. In bouillon flasks the bacilli, at temperatures between 30° and 35° C., after twenty-four hours, show a finely flocculent whitish, slowly increasing sediment. During the next twenty-four hours the flocculi extend upwards from the bottom along the walls. A fine whitish ring of growth then forms on the surface and in course of time covers it. If the flask is protected from any motion and kept perfectly undisturbed, bands and strands of bacilli finally grow downward from the surface membrane. The contents of the flask now present an appearance which somewhat reminds one of stalactite and stalagmite formation A slight jar sends the stalactites to the bottom and destroys the characteristic appearance. The stalactite formation can be assisted by floating on the surface of the bouillon a few drops of an indifferent substance, such as butter, olive or cocoanut oil, small pieces of cork, splinters of wood, etc. Of course, these bodies should be added to the bouillon before sterilization. The bouillon used in connection with our plague work has been one of a fixed degree of alkalinity recommended particularly by Kossel and Overbeck as most favorable for the development of the plague bacillus. Such a bouillon is prepared in the ordinary manner and alkali is added until the broth is neutral to litmus, then 0.5 gram of crystallized soda is added to each 1,000 cubic centimeters. In bouillon cultures the plague organism has a marked tendency to form longer chains, which are composed of ten to twelve or even more small, short bacilli. Such chains on first sight appear much like streptococci, but a more careful examination reveals their proper structure. The individual links of the chain are not arranged in a perfectly straight line, but angles and bends are frequently seen.

The following facts may be added to the enumeration of the cultural peculiarities of the plague bacillus: It does not liquefy gelatin or blood serum, does not ferment dextrose, levulose, lactose, or mannit, grows sparingly on potato and on milk, which latter it does not coagulate.

In the preceding description of the morphology and of the cultural properties of the plague bacillus no attempt has been made to bring out all the details which have been reported in an extensive literature, but merely to state clearly and emphasize those points the knowledge of which is indispensable in the bacteriologic diagnosis of plague. Staining of the plague bacilli in sections will be referred to later.

NOSOLOGY AND CLASSIFICATION.

Bubonic plague of man is an acute, occasionally more subacute, infectious disease, caused by a specific micro-organism, Bacillus pestis, which generally first gains entrance through a trauma of the skin or the mucous membranes and thence finds its way into some of the peripheral lymph glands. In other cases it is inhaled into the lungs, or in still others it enters directly into the general blood circulation. These different methods of infection at once suggest a classification into three main types—bubonic plague proper, plague pneumonia, and plague septicæmia. Perhaps the most prominent and most constant—certainly, even on superificial examination, the most manifest-pathologic feature of all forms of plague is the occurrence of hemorrhages both local at the site or sites where the plague bacilli are colonized in great numbers, as in the lymph glands and in the lungs, and general, subserous, submucous, parenchymatous, and interstitial hemorrhages. great frequency and constancy of these hemorrhages in plague has led to its classification as a hemorrhagic septicæmia. Nothing, as we expect to be able to demonstrate, could be more false than such a definition of plague in man. It may be quite true that experimental plague in certain of the lower animals is indeed generally a hemorrhagic septicæmia, but this, of course, a priori proves nothing as to man. Anthrax, in some animals, is undoubtedly a septicæmia, yet usually when contracted by man it is primarily a local disease and frequently remains local. Diplococcus pneumonia, when introduced experimentally into mice, rabbits, or

guinea pigs, produces a septicæmia, yet in man, under natural conditions of infection, it generally leads to a local infection of the lungs—lobar pneumonia. We have in another previous bulletin¹ called attention to the fact that all observations made on men show that the plague bacillus is not present at all early in the course of the disease in the general blood circulation. Our histologic examinations have further demonstrated that as a rule plague bacilli are either found not at all in the vascular system or are present in such very small numbers that an agonal or post-mortem invasion suggests itself.

It appears reasonable to limit the classification "septicæmia" to those infections in which a multiplication of the infecting microorganisms obviously takes place in the general blood circulation. During the last few years a valuable diagnostic method of blood examination in acute infectious disease has been used quite extensively. Its results, however, have led to a misrepresentation of the character and classification of some diseases. This method is practiced as follows: From 1 to 5 cubic centimeters of blood, for instance, in a case of pneumonia or typhoid fever, are drawn from a vein and introduced into a culture flask containing from 50 to 200 cubic centimeters of nutrient broth. If now a pneumonococcus or typhoid growth develops, it is concluded that there is in this case a general infection of the blood. But what does it really mean if 1 to 5 cubic centimeters of blood do contain a few bacilli which develop and multiply under the most favorable artificial conditions, conditions entirely different from those which prevail in the live, undiluted circulating blood? It certainly does not mean that these same micro-organisms could and would multiply in the circulating blood in the presence of antibodies and of many intact leucocytes. As it would be wrong to classify typhoid fever as a septicæmia, because typhoid bacilli enter the blood from the internal lymphatics, so it is wrong to classify ordinary bubonic plague as a septicæmia because some bacilli invade the blood from the infected lymphatics. If we do want to take cognizance of the frequency and of the extent of the hemorrhages in plague, we may define it as an infectious disease with general hemorrhagic toxæmia.

It was necessary early to enter into the discussion of this point

¹Herzog and Hare: Does Latent or Dormant Plague Exist Where the Disease is Endemic? Bulletin B. of G. L., Biological Laboratory, No. 20, I.

in order to explain and justify our opposition to the classification of plague in man as a general hemorrhagic septicæmia.

A study of the histopathology of plague suggests the following pathologic classification into a number of groups, viz:

- (1) Primary uncomplicated bubonic plague.
- (2) Primary bubonic plague with secondary septico-pyemia.
- (3) Primary bubonic plague with secondary plague pneumonia.
- (4) Primary plague pneumonia.
- (5) Primary plague pneumonia with secondary septico-pyemia.
- (6) Primary plague septicemia.

This classification does not, of course, include any secondary or tertiary complications due to micro-organisms other than the plague bacillus. We shall be able to illustrate all of these different types by cases investigated.

A number of writers have distinguished intestinal plague as a special form of the disease. Wilm, for instance, believes that the plague bacilli frequently enter the body of man through the gastro-intestinal tract and thus lead to a type which is well characterized both clinically and anatomically. Hossack says that 5 per cent of the plague cases occurring in the Calcutta epidemic of 1900 were of the intestinal type, and that 3 per cent of those in the one of 1896-97 were of a similar nature. Zupita reported a case of intestinal plague, but from its description it clearly appears that it was one of primary inguinal bubo, with primary buboes of the second and third order in the pelvic and abdominal cavities. fact, we have failed to find in literature a single case which anatomically could be clearly classified as a case of intestinal plague. In our work special attention has been paid to this subject and every one of our cases in which a post-mortem examination was made was carefully examined in order, if possible, to find a primary bubo of the first order of the mesenteric or other intestinal lymph glands. But none was encountered. It appears that up to date the occurrence of intestinal plague in man has not been proved beyond dispute. Even where rats have been fed on plagueinfected food, the disease is generally of the bubonic type (cervical or submaxillary bubo) with simultaneous or secondary plague septicæmia.

Another type of the disease which has been grouped separately, but which generally is not accepted as a type per se, is skin or

cutaneous plague. In our material we have not seen an example of this type, and it may be mentioned that cases classed as cutaneous plague have generally only been observed clinically and not studied post-mortem.

Musehold, in reviewing the literature on cutaneous plague, says:

The term cutaneous plague applies to those cases in which there exists at the beginning a (primary) plague vesicle, pustule, or carbuncle without marked involvement of the lymph glands. If in the further development of the disease the skin lesion does not spread, but the neighboring glands become more prominently involved, then it would be better to speak of cutaneous and bubonic plague, or still more preferably of bubonic plague with primary skin lesion.

The German Plague Commission among its clinical material had fourteen cases of cutaneous plague, all of which, however, were complicated by buboes; and the Austrian Commission likewise saw no case of this form without typical buboes. Only Kitasato has mentioned one case of primary plague carbuncle without buboes but with septicæmia. From these data it would appear that a separation into a group of those plague cases which show a marked reaction at the cutaneous portal of entrance of the virus is hardly justifiable and somewhat arbitrary. The extensive report of the Indian Plague Commission does not recognize a distinct cutaneous form of the disease.

Another type mentioned by writers is ambulatory plague or pestis minor. While such a group is admissible from a clinical standpoint, it has no proper place in a classification based strictly upon the pathology of the infection. Under pestis minor are classed those mild cases in which a swelling of some of the external lymphatics is generally observed, but in which there are no urgent clinical symptoms, and in which, in fact, a correct diagnosis may be arrived at only after more serious cases have made their appearance. These cases properly belong to the bubonic type. Our knowledge of them, even from a clinical standpoint, is very meager; from a pathologic one it is practically nil. While every case of pestis minor is ambulatory, every case of ambulatory plague is not necessarily one of pestis minor. Indeed we can report such a one in which the individual died very suddenly and where on post-mortem examination such profound plague lesions were found, quite aside from its fatal termination, as to remove it from the group of pestis minor.

THE MORBID ANATOMY AND HISTOPATHOLOGY OF PLAGUE AS DESCRIBED IN LITERATURE.

The number of articles on plague which have appeared since the discovery of the specific micro-organism is very considerable. Most of the literature deals largely or exclusively with the clinical aspect, the bacteriology, the prophylaxis, or the serumtherapy of the infection, so that the number of contributions to its morbid anatomy and histopathology is comparatively limited. It is surprising to find, from a lecture delivered by Virchow in 1879, how little was then known of the pathology of the greatest scourge of the Middle Ages.

Virchow had no opportunity personally to study cases of the disease, and he therefore simply gives a review of the writings of the eighteenth and nineteenth centuries, up to 1879, drawing attention to the then prevalent contradictory views on the pathology of plague. From these he selected, in an admirably critical manner, those which indeed come nearest to the correct pathology as it is now known. Virchow's plague lecture in 1879 also contains this remarkable prophetic declaration: "To me the similarity of anthrax and plague is so great that I consider it very possible that we shall find an organism as the carrier of plague infection. However, an attempt to find it has heretofore hardly been made."

Aoyama, simultaneously with the bacteriologic work which led to the discovery of the plague bacillus, investigated the gross and microscopic pathology of the disease. He described the swelling of the infected lymph glands as brought about by a proliferation of their cells and he noticed the changes found in plague in the periglandular tissue. He stated that the spleen was always considerably enlarged, hyperemic, and soft, the kidneys and the liver somewhat increased in size and much congested, while the cells of these organs showed parenchymatous degeneration. The lungs are described as unchanged. The plague bacilli were found to be present in large numbers in the affected glands but rare or absent in the blood.

Yamagiwa's observations were made during the latter part of the plague epidemic in Formosa in 1896. While he had access to a considerable number of cases which he could study clinically, his pathologic material, owing to political reasons, was limited to three post-mortem examinations. Besides these he was able to obtain several glands extirpated inter vitam which he included in his microscopic studies. All three of Yamagiwa's cases were bubonic plague, one complicated by a metastatic dissemination of the bacilli in the lungs, spleen, and liver, or, as we would now classify it, a primary bubonic case with secondary plague septico-pyemia. However, the summary of the morbid anatomy and histopathology, as given by Yamagiwa, while quite correct on the whole, does not apply to all cases of plague, as the investigation of a larger amount of material very clearly shows.

At the time of Yamagiwa's writings plague pneumonia had not yet been described by modern writers, nor was plague septicæmia well understood. So the statement that all changes, except those of the lymph glands and the tissues of their immediate neighborhood, are generally insignificant, is untenable. We have among our own cases, some of pneumonic and septicæmic plague, in which the changes in the glands are quite insignificant compared with those in other organs, and of course this observation is at present not at all new.

It is very interesting to note that in Yamagiwa's third case, where the hemorrhagic edema and the swelling of the cervical and submental glands was enormous, and the consequent pressure upon the trachea very great, the latter suffered an incomplete fracture of its cartilages and became flattened out into a scabbard-like canal. The frequency and importance of submucous and subserous hemorrhages in plague were duly emphasized by Yamagiwa, and he calls attention to the fact that in the affected lymphatic chain the gland nearest to the point of entrance of the bacillus is more profoundly changed than are those farther away. However, this is not always the case. Other changes particularly mentioned are parenchymatous nephritis and cloudy swelling of the liver and heart muscle, acute swelling of the spleen, and edema of the lungs. The main histologic alterations described are degeneration of the renal epithelium with granular material in the tubules, cloudy swelling of the liver parenchyma cells, dilatation of splenic vessels with hemorrhagic infiltration, necrotic foci and the presence of plague bacilli, dilatation of pulmonary vessels with interalveolar blood extravasation, vascular dilatation, phlebitis and necrosis of the lymph glands and of the periglandular tissue. The extensive blood extravasation in the affected glands is explained in the following words: "The profound change in the walls of the veins, the great loosening (Auflockerung) of the substance of the vessel walls in consequence of cellular infiltration, brings about colossal blood extravasation inside and outside of the lymph glands."

The credit of having first lucidly and correctly described a form of plague which is now universally recognized as a separate and important type—plague pneumonia—belongs to Childe of the Indian Medical Service. Since his description of the pathology of plague pneumonia has not been given the prominence it well deserves, by several writers, it will here be quoted somewhat at length. Of course reference to a pneumonic form of plague had been made long before Childe's publication.

In fact, it had been well observed during the Middle Ages. During the great plague pandemic which decimated Europe between the years 1347-1350 pneumonic plague was very prevalent and Guy de Chauliac, the physician of Pope Clement VI, who observed the plague in Avignon, and who himself became very sick with it, distinguished two types and wrote: "Pestis habuit duos modos. Primuis fuit per duos menses cum febre continui et sputo sanguinis. Et isti moriebantur infra tres dies. Segundus fuit

per residuum temporis cum febre etiam continua et apostematibus et anthracibus in exterioribus, potissime in subasellis et inguinibus. Et moriebantur infra quinque dies. Et fuit tante contagiositatis, specialiter quæ fuit cum sputo sanguinis, quod non solum morando, sed etiam inspiciendo unus recipiebat ab alio." (Haeser: Lehrbuch der Geschichte der Medicin, 3 Aufl., Iena, 1882.)

Childe gives the following description of the first case in which he made a post-mortem examination: "The lungs showed much general engorgement and ædema, with sero-sanguinous frothy fluid in the bronchi, but no pus; the usual appearances of acute bronchitis were absent. There was one small pneumonic patch the size of a walnut, in the early second stage, situated below the apex on the front of the right lung, and two similar but smaller patches at the same part of the left lung. These patches stood out a little from the surface, and were airless, friable, sank in water, each was surrounded by a dark ring of engorgement, which merged into the healthy lung, and there was recent pleurisy over the pneumonic areas. All the other organs were examined and showed considerable engorgement, but no special lesion was observed. The cervical, the axillary, and the lumbar lymphatic glands were slightly enlarged. The left illiac slightly enlarged, red, and soft; all the other glands, including the bronchial, looked absolutely normal. Cultures made from the pneumonic lungs and spleen developed plague bacilli." Childe, in the same article, reports the clinical features of two cases of pneumonic plague—those of a physician and of his nurse-and then says:

"This form of plague is highly infectious, and probably has a large share in the spread of the disease, for in these cases the patient's sputum is practically a pure culture of the plague bacillus, and, as there is reason to believe that many of the cases are not recognized as plague at all, precautions are not taken by the patient's friends and the dangerous nature of the disease is not appreciated. I have no means of knowing how frequent this variety of plague has been in the present epidemic, but there is some evidence to show that a considerable number of cases have occurred. * * With regard to the literature on this subject, I have not been able to find a published description of this variety of plague, but an allusion is made in the accounts of the Pali epidemic of 1836, and it is stated that the Astrakhan outbreak of 1877 was first regarded as croupous pneumonia or as typhus complicated by pneumonia. From the reports on the Hongkong epidemic it appears that plague pneumonia did not occur there. There is just this to add: The usual definition of plague in works of medicine is: 'A specific fever, attended by bubo of the inguinal or other gland,' but it seems that such a form of words does not include all varieties of the disease."

In a later contribution on the pathology of plague Childe also takes up that of the common bubonic type, and states that the glandular lesion is chiefly at the site of the bubo and there are but slight lesions of the lymphatic glands throughout the body, but there are found engorgement or petechiæ, or hemorrhages in nearly all viscera, notably in the alimentary canal, especially the stomach and large intestine,

the kidneys, and bladder, the pericardium, pleura, and peritoneum, and with enlargement of the liver and spleen. In the septicæmic form there is general involvement of nearly all the lymphatic glands and lesions in the other organs similar to those found in the bubonic form. No bubo of the mesenteric glands was ever found. These glands were always examined, and, though changes might be found in them, they were always less marked and less distinct than plague glands found in other parts of the body. In short, there was no necropsy, which went to show that the plague bacillus had reached the stomach or intestines—for example, in food—and thence infected the mesenteric glands.

In his later contribution Childe describes the pneumonic form of plague as follows:

"In this form of plague the only marked evidences of disease are found in the lungs. The lymphatic glands and other organs are scarcely affected at all. In the lungs there was general engorgement, with considerable ædema, a reddened condition of the mucous membrane of the bronchi, but no marked evidences of bronchitis, and frothy watery fluid, sometimes blood stained, could be squeezed from the bronchi. (Pus in the bronchial tubes was only found on one occasion.) A number of pneumonic patches were found scattered through the lungs, varying in size from a pea to an egg. They were light pink or red gray in color, solid, airless, and sank in water. They were rounded in shape, and usually separated by a distinct ring of engorgement from the crepitant lung around. Some, instead of being pink, were of a deep blood color throughout and less solid, and some of these had a small, greyish, more solid center. Those of the patches which were situated on the surface of the lung were prominent, and projected distinctly from the surface whilst the pleura over them was roughened, and showed signs of early inflammation. These patches had, in fact, the appearance of the first and second stages of lobular pneumonia, but no patches were found which had passed on to the third stage of softening and breaking down. In a few cases larger masses of pneumonic lung than these were found and once about half the lower lung was found in this condition. Petechial hemorrhages were usually found on the surface of the lung; the bronchial glands were either enlarged, swollen, ædematous, soft, and distinctly engorged, or else they were small, and of the usual appearance, perhaps a little engorged. The remaining lymphatic glands throughout the body showed none of the appearances of either the bubonic or septicæmic form of plague; most of them looked absolutely normal, and the only noticeable change was that the axillary and sometimes the cervical chains were a little engorged."

Childe found large hemorrhages absent in plague pneumonia, but the usual subserous or submucous petechiæ and ecchymoses were present. This author describes the microscopy of the pneumonic form as follows:

"A section of lung tissue, apart from a pneumonic area, shows great engorgement of all large blood vessels, and of the alveolar capillaries as well, and patches of hemorrhage into the alveoli around these engorged vessels are seen scattered about. In a pneumonic area three zones can be made out. At the circumference there is intense engorgement of

all vessels including alveolar capillaries, the alveoli are full of blood and the hemorrhage is so intense that many of the alveolar septa are broken down, entirely absent, or represented by mere shreds. Within the circumference is seen a zone in which the alveoli are intact and are completely filled with well-stained cells, so that there is no interval between the alveolar wall and their contents, and in the center is one universal mass of similar cells, and the cellular infiltration is so extreme that the walls of the alveoli are scarcely visible. Such is the general arrangement of the pneumonic patch, although there may be alveolar hemorrhage in parts of either the middle or central zone. Under a higher power the alveoli of the circumference are seen to be completely filled with blood corpuscles, and a little fibrin or none at all, whilst the dense central mass of cells consist of catarrhal epithelium and leucocytes with some granular débris. Thus the pneumonic area has the appearance of very extreme lobular or catarrhal pneumonia. The walls of the bronchial tubes, as well as the large veins, show great engorgement and there are hemorrhages into the vein walls. Blood and catarrhal cells may be seen in the finer bronchi, but the bronchial mucous membrane is scarcely altered, there being at most a little cellular proliferation. There are the appearances of acute pleurisy over those pneumonic areas which project upon the surface of the lung, with hemorrhages beneath the pleura. The bronchial glands show engorgement of blood vessels, but in some cases these conditions are only slightly marked and the glands looked nearly normal.

"In cases of plague pneumonia the bacilli were seen in abundance in the pneumonic areas; they could be found in profusion amongst the catarrhal epithelial cells and leucocytes which filled the alveoli and terminal bronchioles, as well as among the blood corpuscles of the alveoli into which hemorrhages had occurred."

Childe also describes a case of plague septicæmia with secondary deposits in the liver, which in this particular instance was most peculiar; it was slightly enlarged and congested, as in the early nutmeg condition, and was stuffed throughout with small, yellow, rounded masses, varying in size from a pin's head to a pea. They were rather soft and friable, but not fluid, and there was no area of engorgement around them. They were found both on the surface and throughout the whole substance of the liver. They looked like necrotic foci and microscopically proved to consist of dense masses of plague bacilli with necrotic cells surrounding them.

It may here be mentioned that Bazaroff, working under the direction of Roux, first produced plague pneumonia experimentally in animals by introducing plague bacilli in their nasal cavities. The disease developed by experiments is described as a lobular or confluent broncho-pneumonia with secondary general septicæmia.

The German Commission distinguishes three types of plague: Bubonic plague, plague pustule of the skin, and plague pneumonia. Whether an intestinal form of plague exists the Commission is unable to decide; it did not encounter a case in the human material examined, though it succeeded in producing intestinal plague in rats and monkeys by feeding

them infected food. The Commission does not believe in the existence of a true primary plague septicæmia and its report states with reference to this subject:

"Primary plague septicæmia probably does not exist. At least our own Commission as well as the Austrian one, and other investigators, have found on post-mortem examination, in such cases in which the portal of entrance of the virus could not be ascertained, small hemorrhagic glandular foci, or a focus in the lung. These had in consequence of the indifference of the patients or in consequence of their occult location, escaped notice during life. Hence plague septicæmia is not a special type of the disease, but the generalization of a primarily local process. That it may then again lead to other secondary internal foci we have demonstrated in a case of plague meningitis."

Septicopyemic processes with pus metastasis, the commission believes to be due to a mixed infection, as is also the case in purulent abscess formation of the plague bubo. From this condition the commission separates a puriform softening of the bubo without abscess formation, which may occur in pure plague infection without the presence of other micro-organisms. The pathologic anatomy is described as follows:

The bubo is anatomically a larger or smaller tumor, which contains one or more enlarged lymph glands; these are rarely greater than a pigeon's egg. They are united into one mass by either ædematous or hemorrhagic connective tissue. The glands and the surrounding tissue show all degrees of inflammation, from simple medullary swelling to ædematous infiltration, bloody infraction, suppuration, and complete necrosis, according to the intensity of the process, the duration of the disease, and the single or multiple microbic infection. As is the case with the periglandular tissue, the neighboring fascia, the areolar tissue, the muscles, the sheath of the vessels and the nerves may likewise be included in the edematous gelatinous or hemorrhagic infiltration; these structures may, as it were, become parts of the bubo. It is not so rare to find a bubo which extends from the inguinal glands to the cysterna chyli, from a cubital gland into the axillary space up to the vena subclavia or from the angle of the maxillæ deep into the thoracic cavity. In such extensive cases the peripheral glands show a milder, the more central ones a more profound degree of inflammation and destruction; while the younger stage of the process is present in the latter, the older one in the former. The anatomical findings in the plague-infected lung can be described with few words. In the lobular form, we generally have quite an extensive process with its favorite seat in the lower lobe. This lobular type of pneumonia is characterized by a peculiar mixture of the different stages of hepatization and by an accompanying serous catarrh. In the hybrid types in which old tubercular foci and fresh plague inflammatory processes are mixed, the picture becomes still more varied. Twice we saw in croupous foci, necrosis and hemorrhagic infiltration of the center to such an extent that expulsion of larger masses

of lung tissue and profuse hemorrhages might have occurred at any moment. The bronchial glands, in some cases of plague pneumonia, appeared like the external primary buboes, while in other ones, more extensive changes were lacking. Besides the primary lesion one regularly finds in plague cases, even in those which have died in the second or third week from complicating affections, blood extravasations into the various internal organs. Rarely have such hemorrhages taken place into the skin, the subcutaneous connective tissue, or into the muscles. However, petechiæ in the mucosa of the intestinal tract, frequently confined to the fundus of the stomach and to the cecum, are often found. These hemorrhages usually vary from the size of a mere point to that of a lentil, or sometimes they may even be larger. They often have become confluent stripes, and appear as such on the mucosal folds. In a few cases such punctiform or linear hemorrhages are seen from the pharynx to the anus. Where old ulcerative processes are present the hemorrhages frequently appear around such lesions. One finds petechiæ regularly in the pelvis of the kidneys, more rarely in the renal capsules, in the gall bladder, in the serous membranes of the heart, the lungs, the liver, etc. The lungs, the testicles, the sheath of the nerves, the dura, the uterine mucosa, the placenta, are in some cases the seat of more extensive hemorrhages, even if far distant from the seat of the primary bubo.

All these hemorrhages are not the direct effect of the bacteria, but more probably the consequence of intoxication. In proof of this view the Commission reports that it obtained three fetuses from plague patients. All three presented numerous hemorrhages but were found absolutely germ free. The Commission further states that it generally found marked parenchymatous degeneration in the internal organs, generally enlargement of the spleen and swelling and hyperemia of the lymph glands aside from those affected by profound hemorrhagic infiltration.

The German commission does not report any microscopic examination of tissues from plague cases.

Albrecht and Gohn,¹ of the Austrian Plague Commission, have introduced the terms primary and secondary buboes. Primary buboes, according to them, are those which are formed by the introduction and the extension of the micro-organisms along the lymph channels, while a secondary bubo is always the result of the propagation of the virus by the blood current. The chief form of plague is denominated by them "septicæmic hemorrhagic." It is characterized by a primary bubo, located most frequently in the inguinal, axillary and cervical regions, about which there occurs a hemorrhagic ædema, and at greater distance hemorrhages into the organs. The spleen is swollen, the general lymph glands are enlarged, and the parenchymatous organs show degenerative changes. The primary bubo may be entirely lacking, the swelling of the lymph glands may be inconspicous, and the hemorrhage may be the only pronounced sign.

¹ Albrecht and Gohn's original report has not been accessible, and we therefore quote mostly from Musehold, Flexner, and others who have freely referred to the work of the Austrian commission.

A second form of the pest which they describe is the septicopyemic. In it embolic foci are present in the lungs, the liver, and the kidneys. third one is the primary pest pneumonia, which is a confluent lobular pneumonic process, usually attended with noticeable lymphatic enlargement. The common portal of entrance of the bacilli is the skin, but only exceptionally can the precise point of infection be discovered, the demonstration of the place and the mode being indicated by the location of the primary bubo. A single instance of purulent meningitis, due to the pest bacillus, was encountered by the Austrian Commission. The invasion of the blood takes place always from the primary bubo or from the lungs, as a primary blood infection does not exist. The primary bubo may be so small that it cannot be discovered clinically and is found anatomically only after careful and prolonged search. In foudroyante cases the formation of a bubo may fail altogether. The primary bubo is distinguished by destruction of the lymphatic parenchyma, necroses, and hemorrhages, and (in the case of mixed pyogenic infections) by suppuration. A similar condition is found in the surrounding cellular tissue. Bacilli are present in great numbers. The secondary buboes show, on the other hand, uniform hyperemia, occasionally hemorrhages, and, in more protracted cases, medullary swelling. The primary buboes of the second order approach one or the other type, depending upon their distance from the gland primarily affected. The multiple hemorrhages are not the result of the action of toxines elaborated at a distance, but are caused directly by bacilli which may always be found in the neighborhood. Parenchymatous and fatty degeneration of the heart, the liver, and the kidneys occur commonly. Splenic tumor is a constant occurrence. The spleen shows marked changes. The pulp is distended with blood and contains many poly-morpho-nuclear cells. The follicles are but little altered, but the trabeculæ are swollen and homogeneous. In some instances the endothelial cells of the pulp have proliferated and desquamated. When very great numbers of bacilli are present miliary necroses may occur. The bacilli are very abundant in the pulp, more scanty in the follicles; sometimes they may be contained within cells. In the pyemic form of the disease foci are found in the liver, the lungs, the kidneys, the spleen and the musculature. In about one-third of all the cases of plague secondary pyococcal infection has taken place.

The primary pneumonia appears in the form of a lobular consolidation, which, when foci are numerous and confluent, may produce lobar solidification. Bronchitis is present. The bronchial lymphatic glands present appearances of primary, the others of secondary buboes. In addition to the primary, two other forms of pest pneumonia are recognized, one metastatic, which appears as multiple and larger foci, seated beneath the pleura, the other as an aspiration pneumonia, from the aspiration of infected material derived from buccal buboes. In plague pneumonia one generally sees shining through the lungs, fine, yellow and red markings or spots, which are produced by yellowish points or stripes on a red background. The picture resembles the one presented by plague lymph glands rich in bacilli. In the lungs this appearance is produced by enormous masses of bacilli contained in the alveoli. The cut surface shows a picture

similar to that seen on the pleural surface; much viscid exudate can be scraped from the surface. The plague foci in the lungs are surrounded by a hemorrhagic ring in an ædematous area. The alveolar septa are broadened. Microscopically they consist of a tissue which stains well with eosin, and which contains a few cells or cell nuclei or red blood corpuscles. The finer changes in the lymph glands, according to Albrecht and Gohn, consist in necrosis of the vessel walls and in a very peculiar coagulation of blood constituents. The more resistant vessels are generally enlarged, their walls somewhat thickened. They are either uniformly thickened and homogeneous or more frequently composed of solid bands of fibers which stain deeply with eosin. In the interior of a vessel with such changed walls, one finds a similar reticulum, sometimes consisting of fine filaments, sometimes containing irregular masses or lumps. Threads penetrating the vessel walls are in connection with the threads in the interior. The picture reminds Albrecht and Gohn of the coagulation necrosis observed in the epithelial layers of a diphtheritic membrane. Fibrin, however, is either not found at all or else sparingly.

Wysokowitz and Zabolotny, members of the Russian Plague Commission, studied the disease in Bombay; they made twenty-seven autopsies and most of their cases were those of bubonic plague. Six were of the primary plague pneumonic form. In the bubonic cases they also found all the other glands more or less swollen; however, these were less affected than the primary bubo, which was generally found to be very hemorrhagic. Plague pneumonia is classified by them as a bronchopneumonia, with a tendency of the foci to become confluent in the more protracted cases. A whole lobe was never found involved in the pneumonic process. Hemorrhages in the gastric and intestinal mucosa were present in many cases of all types, and the mesenteric glands were usually enlarged; in one case the liver showed necrotic foci. The Russian authors, neither in their clinical nor in their post-mortem researches, saw a case which would suggest the intestines as the portal of entrance of the plague virus.

The Anglo-Indian Plague Commission, whose medical members were Fraser and Wright, has published in five volumes the most extensive report on the great modern plague epidemic of India. It divides plague into four types, namely, (1) bubonic, (2) septicæmic, (3) pneumonic, and (4) pestis minor or ambulans, and elaborates upon the classification as follows (Vol. V., p. 53):

(1) Plague, in its most typical variety, takes the form of bubonic plague. In this form of plague the bacteria are carried by the lymph stream into the lymphatic glands, in particular into the lymphatic glands of the groin and the axilla—less frequently into those of the neck. The bacteria multiply there, and excite a considerable amount of swelling and inflammation, which manifest themselves in the form of a bubo. The development of this bubo is often associated with severe pain. Following closely upon—more rarely preceding—the development of the bubo, constitutional symptoms set in. They consist in malaise, headache,

and fever, frequently accompanied by collapse and vomiting. When the bacteria have grown through the meshes of the lymphatic filter, they are carried on by the lymph stream into the blood. The disease then becomes septicæmic.

- (2) Distinguishable clinically though, from the point of view of the pathologist, not sharply marked off from the secondary plague septicæmias just described, are the cases of plague commonly spoken of as septicæmic, in contradistinction to bubonic cases. These are the cases where, owing to the more rapid passage of bacteria through the lymphatic filter, and possibly to a greater production of bacterial poisons, the constitutional symptoms precede and overshadow the local symptoms, the disease being in most cases rapidly fatal.
- (3) In the third form of plague, first bacteriologically established by Captain Childe, I. M. S., plague bacilli invade the lungs and give rise to pneumonia, death occurring in most cases within a few days after the patient has been attacked. Owing to the absence of buboes, the pneumonic form is often classed, along with the septicæmic form, in the category of nonbubonic plague.
- (4) In addition to the three main types of plague which have been described above, an abortive form of bubonic plague comes under observation. This is technically known as pestis minor, or pestis ambulans. It can not be doubted that in these abortive bubonic cases the bacteria are, as in the case of ordinary bubonic plague, carried to the lymphatic glands, but they are held back there, the disease stopping short of the septicæmic stage. In correspondence with this the constitutional symptoms are very light. Indeed in certain cases not only the constitutional, but also the local symptoms may be so slight as to be, except for their pathological interest, almost undeserving of attention. Such cases appear to be extremely common among persons who have been much exposed to the infection of plague and are characterized by sensations of numbness and tingling, or by neuralgic pains, which in many cases are associated with the development of spotty glands in the armpit and the groins. We may, however, remark here that the whole question of pestis minor urgently requires to be more fully elucidated.

From very extensive data the Indian commission has compiled the following summary of the pathology of plague:

Caste and other prejudices of the natives, which were consistently respected, have limited the number of post-mortem examinations relatively to the large mortality from the plague in India. Descriptions of the post-mortem appearances have, however, been laid before us, which together amount to a considerable total, and are sufficient to clearly establish the more important pathological features of plague. References may specially be made to the reports of Dr. Choksy; Captain Childe, I. M. S.; Dr. Dyson and Captain Calvert, I. M. S.; Captain Thomas, I. M. S., and Captain Leuman, I. M. S., and to the evidence of several of these gentlemen and also of Captain Wilkinson, I. M. S., Major Evans, I. M. S., and Captain Elphick, I. M. S.

Pestis minor.—Death from Pestis minor probably never occurs, at any rate, no description of the pathology of plague deals with this type.

Pestis major, lymphatic system.—(a) Buboes.—The appearance of the buboes was characteristic. As we have already stated, they varied much in size and to a less extent in number. For the most part they consisted of enlarged lymphatic glands surrounded by extravasated blood and serum, which in many instances extended widely and deeply into the neighboring areolar tissue, and often penetrated into the substance of the muscles. Sometimes only one gland was included in a bubo, but more frequently there were several, and at times an external bubo was directly connected with one involving deeper seated neighboring glands, and when a femoral bubo extended inwards so as to be continuous with one involving the iliac glands. The swollen glands and the surrounding tissues were often "matted together-so that it was difficult when once cut into it (the bubo) to be quite sure which was gland and which was surrounding tissue." The affected glands were round or oval in shape, and they varied in size from that of a pea to that of a walnut. On section, they were seen to be much engorged with blood, of a light red or deep purple color, and of firm consistence in some cases, but soft and almost diffluent in others. When the bubo was in the groin, the limb of the same side was usually swollen and œdematous; when in the axilla, the serous effusion and hemorrhagic exudation was sometimes so great as to form a swelling involving the whole axilla and extending down the inner aspect of the arm to the elbow, and down the side of the chest nearly to the lowest ribs; and when in the neck a large swelling frequently resulted, which pressed upon the larynx, trachea and cervical nerves and blood vessels, and thus produced the dyspnæa which was so conspicuous a symptom in those cases. As buboes in the groin and axilla frequently pressed upon or surrounded the large blood vessels in these regions, the edema in the corresponding limbs is readily accounted for.

It has been pointed out by Dr. Childe that in axillary buboes and occasionally in chains of enlarged glands along the course of the iliac artery and veins, the extravasated blood may be continuous with blood in the interior of the veins, thus forming "a direct path for the passage of the plague bacillus from the gland to the lumen of the vein, and so into the general circulation."

In a few cases of *Pestis major*, no pathological changes were found in other lymphatic glands than those in connection with which buboes had formed; but in most cases, many other glands, and especially the internal glands, were affected. The changes in them, however, were relatively slight, for they consisted only of enlargement, and of either moderate or great congestion, a section of the gland in the latter instance showing much engorgement with dark blood. In individual cases, many or few of the glands were affected; but indeed no lymphatic gland, whether internal or external, seemed to escape involvement, for these changes were observed in various groupings, in the bronchial, mesenteric, peritoneal, iliac, lumbar, trochlear, popliteal, and other glands.

Lymphatic vessels.—In most cases, the lymphatic vessels showed no pathological change, and only in a few were they observed to be congested and swollen, and that only close to the bubo.

Alimentary system.—The pharynx and the tonsils were greatly congested, the latter being enlarged and occasionally in a state of follicular inflammation. This congestion usually extended down the esophagus, and in some cases hemorrhages were found in its walls. The stomach was almost invariably engorged with blood, and both minute and considerable hemorrhages were present in the mucous membrane. These hemorrhages have been classified by Captain James, I. M. S., as follows:

"A mild form in which, on holding the coats of the stomach up to the light, the course of the smaller vessels was found to be picked out by extravasations which also formed small arborescent patches on the mucous surface, or perhaps only reddened the edges of the rugæ.

"A severe form in which there were more or less extensive patches of hemorrhage into the submucous tissues or even into the stomach cavity.

"Distinct numerous petechiæ with definite circular outlines and varying in size from that of a pin's head to that of a split pea. In one case the petechiæ were very numerous and covered nearly the whole of the mucous surface of the stomach. The appearance was so striking that it reminded one of the rash seen on the skin in a severe case of purpura."

Similar appearances have been observed in the intestines. Congestions and hemorrhagic extravasations were most frequently seen in the large intestine and rectum, and less frequently in the small intestines. Occasionally Peyer's patches and the solitary follicles were observed to be swollen. The liver and the spleen were enlarged, the latter being sometimes two or three times the ordinary size. Petechiæ were frequently present on the surface of the liver, and its substance was engorged with dark blood, and was soft and friable, indicating degenerative change. The spleen also was generally in a state of acute degeneration, soft and pulpy, and in some cases almost diffluent. On section, it was brick red or deep chocolate brown and always greatly engorged. The gall bladder usually contained bile, and occasionally there were petechiæ on its mucous lining. kidneys were also much enlarged and engorged with blood, and presented the characteristics of acute parenchymatous degeneration. Minute hemorrhages were sometimes observed on the surface, and petechiæ and coagulated blood in the pelvis and calices. Extensive hemorrhagic extravasation has also been found outside the kidneys, which were then found "lying in a bed of blood." The ureters and bladder were engorged, and petechiæ were frequently present on their inner surfaces. Besides engorgement, no important change was observed in the supra-renal capsules or thyroid gland, and the genital organs presented a normal appearance. The peritoneum was considerably engorged, but, above all, hemorrhages, often of large size, were found in many parts, as in the mesentery, in the retroperitoneal connective tissue, on the under surface of the diaphragm, and over the spleen, liver, kidneys, and intestines.

Circulatory system.—The blood was dark and fluid, and gave the impression that it did not coagulate well. The pericardium generally contained

more fluid than usual, which was sometimes blood stained; and minute hemorrhages were found in both its vesical and parietal portions. The myocardium was generally soft with many muscle fibers undergoing granulo-fatty degeneration; and the right ventricle was always dilated while in many cases there was also dilation of the other cavities of the heart. The walls of the larger vessels appeared normal, but those of the veins were engorged, and minute and large hemorrhages were frequently observed beneath the inner coat. Dr. Childe found that when a large vein was included in a bubo, hemorrhage into its walls was constantly seen, so that the extravasated blood in the gland itself, in the areolar tissue outside, and in the walls of the vein was continuous.

Respiratory system.—The larynx was in all cases affected and in a few instances there was hemorrhage beneath its surface. In cases of cervical bubo, ædematous swelling involved the soft structures of the larynx and the aryteno-epiglottidian folds. The bronchial tubes were also congested and swollen, and in many cases contained blood-tinged frothy fluid; but the trachea was merely congested. The lungs were engorged with blood and with serous fluid; occasionally small hemorrhages were found in the lung substance, and when the lungs were cut into much sero-sanguinous fluid escaped. When pneumonia or broncho-pneumonia had occurred as a complication, the pathological changes produced by these diseases were also present. The pleural cavity contained blood-stained or clear fluid. Subpleural hemorrhages, sometimes of large extent, were found on the parietal, visceral and diaphragmetic pleura and occasionally there was evidence of fibrinous inflammation. Hemorrhages were sometimes present in the anterior and posterior mediastina.

Nervous system.—The dura and pia mater of the brain, as well as the coverings of the spinal cord, were congested, and in a few instances extravasations of the blood were found on the dura mater. The brain substance and spinal cord generally presented a healthy appearance; but occasionally congestions and ædema were present, which involved likewise the choroid plexuses. No pathological change has been observed in the nerve trunks.

Bones, joints.—It has further been noted that the marrow of the long bones is red and congested as in other infectious fevers, and that evidence of an infective inflammation is found in the fringe-like processes of the synovial membrane of the knee joint.

Intense or septicamic type of plague.—In those cases in which the plague virus or toxin is in the patient widespread from the beginning of the illness, so as early to produce a general poisoning, whether septicamic or toxamic, the pathological changes, an might be expected, are much the same as in the more severe cases of Pestis major. Some observers, however, believe that pathological differences occur to distinguish this form of plague, and to serve, along with the symptoms, as a justification for the establishment of a so-called septicamic type of the disease. They consist of the absence of buboes having the characters above described, and of a widespread involvement of glands, with distinctive changes in several of them. Although the lymphatic glands are always affected, in place of the affection consisting of one or, more rarely, of several groups of glands

being enlarged and surrounded with sero-sanguineous extravasation, while the other glands are either normal or merely enlarged or congested, in this, the so-called septicæmic form, the affection of the glands shows itself as a general involvement of all, or nearly all, of the lymphatic glands of the body, although in many instances the affected glands were chiefly those of the mesentery. In no case, however, did the involvement proceed to the formation of the characteristic plague buboes, but only to a moderate degree of change, practically restricted to the glands themselves, but still displaying in several of them certain distinctive features. These were moderate enlargement and ædema without much congestion, the glands being pink in color, firm and rounded, and with a soft interior, often possessing here and there small areas of softening surrounded by firm substance. Several of the affected glands may be thus modified, while others of them are merely enlarged and engorged with blood, thus resembling the less affected glands of ordinary Pestis major. Excepting the lymphatic glands, the parts that were affected showed essentially the same pathological changes as in the bubonic variety of Pestis major, but usually the number of parts affected was smaller and the degree of change in them was less.

Pestis pneumonica or pulmonalis, or primary plague pneumonia.—Inflammation of the lungs occurs, as already stated, with considerable frequency during the course of the plague, and it then becomes a serious complication. It also occurs so early in the disease as to justify the belief that the plague virus had affected the lungs either primarily or coincidentally with the more general affection of the body, thus constituting a form of plague which is distinguished as primary pneumonic plague. When contrasted with those occurring in Pestis major, the pathological changes elsewhere than in the lungs are less intense. While those in the lungs are more intense. The lymphatic glands are only slightly affected, and external buboes having the characteristics seen in Pestis major are seldom, if ever, encountered. Congestion and enlargement of organs and even hemorrhage in mucous and serous membranes may be present, but they do not assume the proportions attained in Pestis major. On the other hand, the lungs are conspicuously affected. The whole substance is engorged, the large as well as the small blood vessels being distended; and hemorrhagic zones are seen scattered throughout the lungs, filling the alveoli and often breaking down their walls. Within the hemorrhagic zones are areas in which the alveoli are completely filled with leucocytes, epithelial cells and granular débris, constituting, with the surrounding zones of hemorrhage, blood-congested areas of catarrhal pneumonia. In these areas, as well as in the fluid matter contained in the trachea and bronchi, plague bacilli are abundantly present. Greyish necrotic patches have also been found containing large numbers of plague bacilli. The bronchi are engorged with blood, and catarrhal cells are found in their terminations. Over affected areas at the surface of the lungs, the pleura may be acutely inflamed. In most cases, the bronchial glands were congested, and there was a little hemorrhage into the gland substance; but in some cases, their appearance was normal.

While, however, a catarrhal inflammation of lobular distribution has most frequently been regarded as the characteristic type of primary plague pneumonia, several observers have denied its existence, and have asserted that croupous (lobar) pneumonia is the form 'which most frequently occurs. Major Evans, I. M. S., and Captain Elphick considered that all cases of typical plague pneumonia come under the latter category, and Major Jones expresses the opinion that "lobar pneumonia is common." Major Evans stated that the pneumonia is distributed in small patches, constituting lobular areas, only when the inflammation has not advanced far; but that it is lobular to the extent of involving a whole lobe or the greater part of a lobe when the lung inflammation has advanced further. Captain Elphick, I. M. S., described several autopsies in which individual lobes or even an entire lung were consolidated, and he stated that "every case of pneumonic plague examined showed lobar condensation." It may further be stated that in many cases only slight changes were found in the bronchi. It is therefore possible that the pneumonia is lobular in patients who have died at an early stage of the disease, and lobar in those who have survived to a later period; or, otherwise, that lobar pneumonia occurs when the toxin is most virulent and most widely distributed throughout the lung, and lobular pneumonia when it is less virulent and less widely diffused.

The microscopic examination has mainly shown general dilatation and engorgement of the veins and smaller blood vessels and numerous capillary and larger hemorrhages in almost every structure and organ of the body.

Indian Plague Commission's summary of pathological conditions.—"The distinctive pathological changes produced by the virus of plague would therefore appear to consist of universal dilation and engorgement of veins and smaller blood vessels, with hemorrhages, both minute and of large amount, in nearly every part of the body, and of enlargement of the lymphatic glands, with edema and hemorrhage in the surrounding tissues, generally mainly implicating the external glands, but occurring likewise throughout the body and involving in a series of cases the entire system of lymphatic glands. In the lymphatic glands, the characteristic conditions are largely explainable by vascular changes, and even in the pneumonia of plague, vascular dilatations and hemorrhagic extravasations give a special character to the lung inflammation. In no other infective disease are these features represented, but it is of some interest to note that the vascular changes, and especially the prevailing and characteristic tendency to extravasation of blood in almost every part of the body, are closely reproduced in toxemia caused by the organic poison secreted by the venom glands of several species of serpents such as the black snake (Pseudechis porphyriacus) of Australia."

Flexner, who examined six cases of plague in San Francisco, besides giving a summary of the morbid anatomy, reports more fully on the microscopic changes of the lymph-glands and of the spleen. He states that in the primary bubo the separation into the medulla and cortex has been effaced, that masses of lymphoid cells still exist but that they do not compose typical structures. Remains of cell forms, nuclear fragments,

shadows of red blood corpuscles, and plugs of finely granular structure are seen filling spaces which suggest preformed vessels. Hemorrhages exist in the medullary and cortical parts. The bloodvessels are commonly thrombosed. The thrombi consist of cells, both mononuclear and polynuclear and fibrin. The walls of the smaller vessels often are hyaline and structureless. Fibrin stain shows a fine network of fibrin about the vascular walls, occurring partly within the lumen, partly within the wall, and partly beyond in the perivascular tissues. Larger vessels show instead of the fibrinoid transformation of their walls, destructive and infiltrative changes. The intimal and medial coats contain an increase of cells and many cell fragments. This infiltrative condition is especially marked in the midddle coat. That many of these cells are polymorphonuclear is shown by the bizarre forms and the staining properties of the nuclei. The periglandular structures are extensively infiltrated and usually show necrosis as well. The infiltration is partly cellular, partly fluid, along with which fibrin is perhaps invariably present. The ædema and fibrin are found chiefly in the coarse fibrous septa of the adipose tissue; the cells occupy the meshes of the fat cells. Bacilli are abundant in countless numbers within the swollen glands and in the periglandular tissues. They occur in continuous growth throughout the glands, occupying every available space; they completely occlude many blood vessels or, mixed with definite thrombi, compose a considerable part of the plugs. Moreover the walls of the blood vessels contain masses of bacilli having grown within the vasa vasorum and the lymph spaces. The adventitial coats of veins and arteries are especially rich in such growth of bacilli. In the primary bubbes of the second order the changes are all much less advanced and marked.

In a case of tonsilar infection changes similar to those described in the primary bubo of the first order were observed. The presence of cells resembling plasma cells is mentioned and many of these were observed to be phagocytic. The spleen, in Flexner's six cases, was found moderately enlarged, somewhat diminished in consistency, color deeper than normal. The pulp is described by the author as swollen, the swelling being the result of cellular proliferation, cellular infiltration, and œdema. proliferation especially affects cells closely united with the veins and surrounding the trabeculæ. These cells have reticulated nuclei, placed excentrically, and a fair allotment of protoplasm, taking the blue thionin stain. The cells are often polyhedral rather than round. They have a close affinity to plasma cells. The lymphoid cells are increased, but to a less extent than the cells just described. The vascular and other spaces in the pulp contain an increased number of red corpuscles. In the same spaces occur large cells of two kinds. One is a giant cell with single, lightly staining, reticulated nucleus and a moderate amount of protoplasm, and resembling the large, mononuclear, marrow cell with which it is probably identical; it is not phagocytic. The other is a much smaller cell, three to four times as large as the marrow cells and is highly phagocytic.

The macrophages englobe white cells—both mononuclear and polynuclear—but rarely red blood corpuscles. They occur at times within the larger veins, especially such as exhibit the subintimal cellular proliferation to be described. The polymorphonuclear cells in the pulp exhibit great variation in form and many would seem to have been in a state of active migration when the tissue was fixed. They show great variety of form, such as is seen in actively motile cells, and they would seem to be moving in numbers in the pulp. Fibrin is found among the pulp cells and in the fluid, and many bacilli are present.

The Malpighian bodies are increased in size, this being due to the multiplication of the lymphoid cells, and, to smaller extent, of epithelioid cells. The latter do not occupy the centers of the nodes, but are few in numbers and placed peripherally. Their nuclei are large and vesicular, rarely a cell contains two nuclei. Degeneration of cells is uncommon, very few fragmented ones being visible. Rarely small islands of fibrin are present in the nodules.

The blood vessels show two kinds of change. The arteries, chiefly those of the Malpighian bodies, have hyaline walls; the veins of all sizes frequently show subintimal cellular proliferation. The cells in the intima are mononuclear and more rarely polynuclear elements that form a continuous, although not uniform, investment or appear as isolated projections into the lumen of the vessel. Above these cells the displaced endothelial cells can usually be detected. Bacilli are very numerous, especially in the pulp. They also completely occlude small blood vessels, and within the trabeculæ, probably lymphatics. However, since the richest growths of bacilli are often unassociated with reactions, it is highly probable that they may have taken place post-mortem.

Calmette and Salimbeni made observations on plague during the last Oporto, Portugal, epidemic, and they describe the macroscopic and some microscopic lesions as follows: The buboes may be single or multiple, in most cases they were multiple. They consist of one or more glands of the same region. The former are increased in size in consequence of a hemorrhagic inflammation which extends into the periglandular tissue and sometimes into the overlying skin, producing phlyctenæ which may contain plague and other bacteria. Microscopically one finds cellular débris, masses of chromatin, few leucocytes, and many plague bacilli. In the most profound rapidly fatal cases there are extensive hemorrhages, however, when, death is delayed and is brought about by complications, one finds that the contents of the bubo are true purulent material with few plague bacilli. The authors also described the so-called cutaneous plague type, of which, however, they saw very little in Oporto. Once they observed a case which began as an edematous, very intense inflammation of the skin of the hand and the forearm and progressed to dark discoloration and necrosis. In all three cutaneous cases which were observed a bubo developed in the neighborhood of the superficial plague lesion. Skin lesions in the shape of petechiæ, ecchymoses, pustules, and hemorrhagic vesicles were also observed in typical primary bubonic plague. The two authors observed a case which resembled hemorrhagic smallpox. In the internal organs congestion of the intestinal mucosa, swelling of the mesenteric glands,

fatty degeneration and necrosis of the liver and an enlarged spleen sometimes of good consistency, but more frequently soft and friable, were found on post-mortem examination. The kidneys showed evidence of parenchymatous degeneration, sometimes with hemorrhagic foci; such were also exceptionally seen in the bladder. The heart showed subepicardial hemorrhages; endocardial inflammation or valvular changes were not seen. Once a meningitis and once a meningo-encephalitis was observed. In all grave forms of the disease the lower and posterior portions of the lungs were found hypostatic. A pure case of primary plague pneumonia was not seen at autopsy.

Jennings, in his Manual of Plague, gives a summary of the pathology and morbid anatomy of the disease, in which he states that discoloration, distinct from post-mortem lividity, caused by small or large subcutaneous blood extravasation is almost invariably present in different situations. Papules, vesicles, or pustules may exist, also scabs or unhealthy ulcers. The skin over buboes may show a necrotic appearance, or necrosed patches of skin or large sloughing ulcers may also be present. Diffuse swelling around the buboes occasioned by infiltration is often observed. lymphatic vessels, except those associated with the buboes, are seldom involved; occasionally, however, a more extensive, widespread lymphangitis may be present. The glands may simply be enlarged, congested, or engorged, or they may show a profound hemorrhagic condition. The hemorrhages are often extended into the periglandular tissue and they may infiltrate the whole neighborhood. Glands distant from the bubo are generally swollen, congested, and engorged. Plague bacilli are abundant in the pulp of the affected glands, but generally disappear after marked softening or true suppuration have appeared. In septicæmic plague the glands show very moderate changes only. In the gastrointestinal tract congestion of the mucosa and edema of the visceral walls are generally observed, and petechiæ in the stomach and large intestines, and to a lesser extent in the small intestines, also occasionally more extensive hemorrhages in the submucous tissue of the stomach occur. The solitary and the agminated glands are swollen and congested, but never ulcerated; the retroperitoneal and mesenteric glands are generally in the same condition and often hemorrhagic. The spleen is much enlarged and subcapsular hemorrhages make the surface lumpy and uneven. The liver is increased in size and engorged, and sometimes presents a nutmeg appearance; occasionally yellow necrotic patches are found scattered throughout the organ. Petechiæ are common on the surface. The kidneys are generally intensely imbedded in extravasated blood; petechiæ are common on the surface, also in the pelves and calices; sometimes coagula are found in the former. Glomeruli are engorged, all the blood vessels distended and the epithelium of the tubules generally in a state of parenchymatous degeneration. The genital organs are generally unaffected, but in cases in which abortion has occurred. the subinvoluted uterus and the ovaries are engorged and ædematous. The lungs are generally engorged. Frequently hemorrhages are found in the lung substance or on the surface, scattered over the pleura, or in the mediastina. When the lungs are primarily involved or secondary pneumonia

supervenes, the walls of some of the alveoli are broken down by the severity of the hemorrhages and patches of catarrhal inflammation, varying in size, are scattered throughout the lungs. These patches are surrounded by belts of engorged lung tissue. They may coalesce into larger patches. Sometimes a whole lobe may thus be consolidated. The patches at first are red and later on become gray; they are quite solid and do not float on water. The air cells in the affected areas are filled with an accumulation of epithelial elements, granular débris, cells resembling leucocytes, intimately mixed into a gelatinous mass in which plague bacilli, often in association with diplococci or streptococci, abound. The vessels in general are dilated, the heart sometimes unaffected, more frequently soft, flabby and friable. In all cases the right side of the heart is dilated and contains post-mortem coagula.

A STUDY OF TWENTY CASES OF BUBONIC PLAGUE.

The material which forms the basis of the original work of this bulletin consists of twenty cases of plague occurring in Manila and upon which autopsies were performed by the writer during the period of time from February 19 to September 8, 1904. These cases were without exception fully examined anatomically on the post-mortem table, and also by cultural and histologic methods. In the majority of the cases, in addition, animal experiments were performed with the organisms isolated. One case which was not completely studied has been included, because it showed the hyaline fibrin thrombosis of the glomerular vessels which is so characteristic in plague. ¹

The histologic material was in all cases at the time of the autopsy immediately fixed in Zenker's solution and was subsequently embedded in paraffin and sectioned. In addition, in one case the material was fixed in Flemming's solution. The stains employed in the study of the sections were hematoxylin and eosin, eosin and alkaline-metylene-blue, carmine and Weigert's fibrin stain, dilute carbol-fuchsin and occasionally other stains. Dilute carbol-fuchsin, which we found the most useful dye for smears from the organs, is not very satisfactory in the treatment of sections, stained with a view of studying the distribution of the plague bacillus, nor did

¹The number of cases upon which necropsies were performed during the above-mentioned period of time has been in excess of twenty; but some of them were not considered in this report because of the greatly advanced putrefactive changes, which excluded a satisfactory anatomic and histologic examination, or on account of the late hour in the day in which, for the sake of an immediate diagnosis, the necropsy had to be hurriedly performed, in consequence of which a careful anatomic study became impossible.

we obain any good results with a modified Romanowsky dye. Eosin and methylene-blue is the best for exhibiting the plague bacillus, but in using this stain one must be careful in decolorizing, or else too many plague bacilli lose the stain and fallacious conclusions may be arrived at. This dye, as a rule, fades quickly in the Tropics in an atmosphere saturated with moisture, and hence the sections treated with it must be examined without delay, and in a later reëxamination, recoloring frequently becomes necessary.¹

The twenty cases to be reported below in detail have been classified under the following six groups:

	Cases.
Group I. Primary uncomplicated plague	11
II. Primary bubonic plague with secondary plague septico-	
pyemia	4
III. Primary bubonic plague with secondary plague pneumonia	1
IV. Primary uncomplicated plague pneumonia	2
V. Primary plague pneumonia with secondary plague septico-	
pyemia	1
VI. Primary uncomplicated plague septicæmia	1
Total	20

GROUP I. PRIMARY UNCOMPLICATED BUBONIC PLAGUE.

[Eleven cases.]

CASE No. 1. LEFT INGUINAL BUBO.

[Necropsy Protocol No. 1009. Post-mortem examination July 27, 11 o'clock a.m., about eighteen hours after death, on the body of C. S., a male Chinese, 36 years old, from 217 Santo Cristo. Died July 26 at 4 o'clock p. m.]

Body of a well-developed, fairly well-nourished, male Chinese about 35 to 40 years old. Post-mortem rigidity not very marked, surface quite cyanotic; post-mortem lividity has made its appearance all over the dependent parts and extends in the shape of some irregular patches to the anterior thoracic regions. Some foamy, slightly blood-tinged fluid escapes from the anterior nares. No

¹We believe the reason for the rapid change of the eosin-methylene-blue to be the following: It is impossible completely to dehydrate sections in an atmosphere saturated with moisture. Hence, after a time the water in the sections will dissolve some eosin, and the eosin solution so formed will in its turn dissolve the methylene-blue. Whether this explanation be correct or not, it does not alter the fact continually observed, that sections stained with eosin and methylene-blue during the rainy season in Manila rapidly fade and, to a great extent, become useless for the study of finer structural details and the presence and distribution of the bacteria.

wounds or ulcerations or abrasions are found anywhere on the surface. The left inguinal region presents a swollen area about 6 to 8 centimeters long and 5 to 6 centimeters wide, which is quite hard, somewhat elastic, and nonfluctuating. No individual glands or groups of glands can be mapped out in the swollen area. The skin is here adherent to the underlying tissues. The hard infiltrated area extends upwards into the inguinal canal. Dark fluid blood escapes from the veins upon section of the body. The serous membranes are injected and dull, and all serous cavities contain a somewhat increased amount of slightly turbid fluid. The increase is most marked in the abdominal cavity. The pericardium is smooth and otherwise normal, except as to the presence of a number of small punctiform subepicardial hemorrhages, which are dotted all over the external surface of the heart. All diameters of the heart are about one and one-third of the normal measurements. The wall of the left ventricle shows considerable hypertrophy. The left auriculo-ventricular opening is normal in diameter, the right one easily admitting four fingers. The myocardium is quite soft and flabby, and pale pinkish-yellow in color. Otherwise the heart is normal. The coronary vessels and their branches are much congested. The lungs are normal in shape and moderately inflated. The upper lobes are pinkish-gray, the lower purplishblue in color. The latter much congested and quite cedematous. The mucosa of the bronchi, the trachea, and the larvnx is swollen and reddened and the veins injected. The epiglottis is much injected, and the papillæ circumvallatæ of the tongue swollen. The spleen is enlarged to two to three times its average size; it is somewhat softer than usual. The capsule is smooth and grayishblue in color. On section it is brownish-red. The pulp does not protrude, the cut surface is smooth, and the trabeculæ and follicles are distinct. The kidneys are normal in size, the capsules smooth and grayish-yellow with some purplish-gray. The capsule peels off easily. On section the blood vessels are found to be injected, the tubules light yellow, and the surface as a whole dull. The Malpighian bodies are not distinguishable. The mucosa of the bladder shows a very few small, submucous, punctiform hemorrhages. The prostate, etc., are normal. The adrenals are soft, swollen and brownish-vellow. The serosa of the stomach is much injected, the mucosa in an intensely hemorrhagic condition. Small hemorrhagic spots are found all over the mucosa on the anterior

wall. Midway between the larger and the lesser curvature a number of hemorrhagic spots have become confluent. The hemorrhagic spots can not be wiped off. The lower part of the esophageal mucosa is likewise much congested. The serosa of both the small and the large intestine is congested and dull. The mucosa shows hemorrhagic spots, particularly in the duodenum. The follicles are swollen. Several Ascaris lumbricoides were found in the esophagus. The capsule of the liver is smooth and transparent. the external color is ochre-yellow, the cut surface is fairly smooth and yellow to light brown. The lobules are not enlarged; the connective tissue does not appear increased. The veins contain much blood. The organ, as a whole, is small and peculiarly formed; the left lobe forms merely a small appendix to the right one. weight is 1,210 grams. The length of the longest transverse diameter is 20 centimeters, the sagittal diameter (antero-posteriorly) 9 centimeters, the thickness from above downwards is 12 centimeters. The left lobe measures 3 centimeters from side to side, 9 centimeters from before backwards, and is 2 centimeters thick. When an incison is made over the left inguinal bubo, it is found that the skin is completely adherent to the subcutaneous tissue, fascia, etc. There first escapes quite a quantity of yellow, bloodtinged serum, and the tissue beneath the skin presents a condition of complete hemorrhagic infiltration. The glands have become confluent, their capsules, except that of one, being indistinguishable from one another. The hemorrhagic infiltration and the cedema extend into the inguinal canal. In the left half of the pelvis the tissues are very ædematous, the loose areolar tissue is yellowish and almost gelatinous; and imbedded in this tissue are swollen, softened, hemorrhagic lymph glands. The iliac glands are in the same condition; the retroperitoneal glands at the bifurcation of the aorta into the common iliac arteries, and even the ones farther up along the abdominal aorta, likewise present a similar appearance.

Smears made from the inguinal glands and from the spleen show very numerous plague bacilli. The organisms are most abundant in the glands.

Anatomical diagnosis.—Hypertrophy of the heart; congestion and fatty degeneration of the kidneys; fatty infiltration and degeneration of the liver; hemorrhagic inflammation and hypertrophy of the left inguinal, femoral, iliac, and retroperitoneal glands; hypertrophy, softening, and congestion of the lymph glands

in general; multiple subserous and submucous hemorrhages. Bubonic plague.

Culture tubes inoculated from the inguinal glands developed a typical plague growth.

Microscopic examination.—Glands: The inguinal glands, the iliac, and the retroperitoneal, all practically show the same changes, which, however, are more profound in the lowermost lymph nodes. All glands contain large tracts of tissue in an advanced state of coagulation necrosis; there is much blood extravasation, which extends into the periglandular loose areolar tissue. The latter also shows much infiltration with leucocytic elements. The capsules of the glands are completely loosened by the same type of infiltration. The blood vessels are dilated and engorged, and their walls show hyaline swelling and inflamatory loosening ("Auflockerung"). Weigert's method shows both solid and tubular wall thrombi in many of the vessels of the gland substance proper, in the capsule, and in the periglandular areolar tissue. Much fibrin is also found in the shape of reticular deposits throughout the sections. In some places a continuation of an intravascular thrombus into an extraglandular network is visible. Plague bacilli, freely scattered throughout the sections, are found either in large masses, in small groups, or as single individuals.

In the spleen the follicles are sharp in outline and the boundaries of the pulp spaces indistinguishable. The latter are densely crowded with cellular elements among which the erythrocytes much predominate. Besides the common small mononuclears and eosinophilics, a fair number of large mononuclears with hyaline protoplasm, which stains moderately with methylene blue, are found. Plague bacilli are seen all throughout the sections; however, nowhere are they present in large groups, but thinly scattered here and there. A few threads of fibrin are occasionally seen.

Kidneys: The renal tissue exhibits complete degeneration of the tubular epithelium, with cloudy swelling and fatty degeneration. The degeneration is most marked in the convoluted tubules, while in the straight tubules apparently normal epithelia are found here and there. The uriniferous canaliculi contain a great deal of granular material. The glomeruli show much dilated capillaries; otherwise they present no marked changes. The renal vessels are all much congested. The interstitial connective tissue is quite cedematous.

In the liver we find small interlobular inflammatory foci, composed of small, round cells. The parenchyma cells are finely vacuolated or coarsely granular; some of the nuclei are absent or do not stain. Here and there a small area is found in which all cells are degenerated (areas of focal necrosis). A few plague bacilli are here and there seen in the capillaries, but none in the interlobular inflammatory foci.

The consolidated areas of the lower lobes of the lungs show an enormous dilatation and engorgement of the vessels and alveoli, completely filled with extravasated blood and with desquamated alveolar epithelial cells. The latter contain much dark granular pigmentary matter. Here and there one sees in the alveoli, cocci, a slender bacillus, and possibly a few plague bacilli. Whether the latter are really plague bacilli is somewhat doubtful; certainly, if present at all, they are very scantily represented.

CASE No. 2. LEFT INGUINAL BUBO.

[Necropsy Protocol No. 989. T. C., a male Chinese, from Ilang-Ilang Street, San Nicolas, 29 years of age. Ill two days. Died June 20, 1904. Postmortem examination nine hours after death.]

The body of a slender, rather poorly nourished Chinese. Postmortem rigidity is still well-marked. Post-mortem lividity is extensive. The anterior abdominal wall presents a mottling of greenish discoloration. The tibial region of the left leg shows two shallow, almost healed ulcers. Both the inguinal regions are swollen, particularly the left one. The swelling shades off into the surrounding tissues. On the right side no individual glands can be distinguished, because the whole region is much infiltrated and edematous. On making an incision into this region, a considerable amount of slightly blood-tinged, serous liquid escapes and the entire tissues are found to be diffusely infiltrated with blood. The individual glands can not be distinguished, as they have become fused into one irregular hemorrhagic mass; nor are the capsules, the cortices, or the medullæ of the individual glands distinguishable. No suppurative changes are observed. On the right side the glands are swollen, softened, and highly congested. However, the capsules are well preserved, and the hemorrhagic condition does not extend beyond the glands proper. The other superficial glands are not palpable. On opening the body cavities, a moderate amount of dark, fluid blood escapes from the veins.

The abdominal cavity contains an increased amount of serous,

blood-tinged fluid. The hemorrhagic infiltration of the inguinal region extends through the inguinal canal, into the pelvis, and along the iliac vessels and left ureter upwards. The pericardium contains a normal amount of serous fluid, but it is blood-stained, evidently by post-mortem imbibition. The heart presents a few small subepicardial hemorrhagic areas. The myocardium is soft and flabby, and pink in color.

The beginning of the aorta presents an atheromatous ulcer, surrounded by a calcareous deposit. Otherwise the heart and the great vessels are normal. The pleural cavities contain a moderate amount of blood-tinged fluid. The pleural surfaces of the lungs are dark purple, with some greenish putrefactive areas. The lungs are full of blood and contain but little air. The bronchi, the trachea and, to a lesser extent, the larynx show a congested mucosa. The spleen is enlarged to about twice its normal size. It is steelgray-purplish on the external surface, and brownish-red on section. The pulp is soft. The Malpighian bodies are fairly distinct. The kidneys are normal in size, purplish-blue in color. On section they are dull and decidedly grayish yellow. They are exceedingly soft, a condition probably due to some extent, at least, to postmortem changes. The mucosa of the left ureter is highly congested and shows some hemorrhagic spots, that of the right ureter is congested, but to a much lesser extent. The mucosa of the bladder shows a minor degree of congestion. The liver is normal in size, rather increased in consistency, and dark purplish in color with some grayish-yellow mottlings. It cuts with increased resistance, and the cut surface is vellow brown in color. The liver lobules are increased in size. The gall bladder and its ducts are normal. The duodenum and the stomach show a highly congested mucosa. The gastric mucous membrane exhibits numerous small punctate or somewhat larger, irregular hemorrhagic spots. The follicles of both the small and the large intestine are highly swollen and congested. The suprarenals are swollen, soft, and dark yellowishbrown in color. The pancreas is normal. The abdominal glands in general are all more or less swollen, congested, and softened.

Anatomic diagnosis.—Hemorrhagic left inguinal bubo; general lymphadenitis; multiple hemorrhages into the serous and mucous membranes; congestion and parenchymatous degeneration of the kidneys; congestion and fatty degeneration of the liver; bubonic plague.

A microscopic examination of smears from the left inguinal glands shows innumerable plague bacilli, most of which are oval, or almost spherical, in form, with a narrow peripheral stained margin, suggesting mere empty shells. Smears from the right inguinal glands contain a moderate number of plague bacilli, which are also quite numerous in the juice of the spleen. The heart's blood contains several varieties of micro-organisms in moderate numbers. Among them possibly a very few plague bacilli are present.

Tubes inoculated from the left inguinal bubo and from the spleen developed a typical plague growth.

Microscopic examination.—Left inguinal glands: The finer gland structure is practically completely lost, though follicles can here and there still be recognized to some extent. All of the vessels show great dilatation and engorgement, and the connective tissue at the hilum is increased; toward the periphery tracts of coagulation necroses are seen. Here the cedema is also marked. All throughout the glands there are found masses composed of innumerable, very densely crowded plague bacilli. In sections stained with eosinmethylene-blue, but which have been too much decolorized, the bacillar masses are somewhat stained by eosin and they look simply like ordinary granular material, for which in such improperly stained sections they may easily be mistaken. It is particularly the periphery of the gland which is quite extensively infiltrated with red blood corpuscles. This extravasation extends beyond the capsule and into the surrounding loose areolar connective tissue, where it is mixed with a more or less marked leucocytic infiltration. The parenchyma cells of the glands are small mononuclears and some plasma cells, polynuclears of the ordinary type, and a few eosinophilics. In sections stained by Weigert's method, numerous capillaries and other small vessels are found to be closed by hyaline fibrin thrombi. This thrombosis is generally complete in the smallest vessels only. In the larger ones we see an incomplete thrombosis. The fibrin in the latter is deposited on the intima and leaves a free space in the center of the vessel, which, however, may show an open network of fibrin filaments. The endothelial lining of the vessels, totally or partly thrombosed, is apparently generally intact, although there may be seen places where the endothelia are missing. Such losses are probably due to an artefact. The vessel walls proper do not show any profound changes; however, a minor degree of œdema and even of hyaline degeneration may here and there be observed. No bacilli are seen in the lumina of the vessels, either in those containing thrombi or in those free from them. In the right inguinal glands the most pronounced pathologic change is the increase of connective tissue at the hilum and the great dilatation and engorgement of the vessels. The congestion is greater on the right side than on the left. This is to be attributed to the fact that there are only few bacilli present on the former one, while on the latter they are so numerous as to have a tendency more or less to crowd all of the autochthonous tissue elements. Some few of the vessels in the right inguinal glands show a network of fibrin, however, none complete thrombosis.

The spleen sections show very numerous plague bacilli. However, they are nowhere found in dense masses as in the primary bubo, but very abundantly as single individuals freely distributed between the cells. The boundaries of the follicles are rather indistinct. The pulp spaces contain numerous crowed red blood corpuscles. In a few small vessels tubular hyaline thrombi are seen.

The epithelial lining of the uriniferous tubules of the kidneys shows cloudy swelling and fatty degeneration. These changes are most marked in the convoluted tubules, but the epithelia of the straight ones are likewise much affected. Much granular material is found in all of the tubules. The intertubular connective tissue is edematous. In a number of the glomeruli the capillaries are closed by hyaline fibrin thrombi, while other capillaries are free and non occluded. The thrombi mostly are solid, though some are distinctly tubular with an open lumen in the center. Occasionally one sees a thrombus extending from a Malpighian tuft into an afferent or efferent, or intertubular vessel. Changes of the vascular endothelium of the thrombosed vessels are not demonstrable. through the renal (and also through the hepatic) tissue, fairly numerous large bacilli, which retain Gram's stain, are found. These micro-organisms clearly represent a post-mortem invasion found frequently in Manila in bodies, when the post-mortem examination can not be made immediately but has to be postponed for some time. Plague bacilli are not seen in the renal tissue.

In the liver the parenchyma cells are finely vacuolated; large, coarse vacuoles are not seen. The capillaries are dilated, par-

ticularly those in the central part of the lobules. Here and there a small, interlobular, inflammatory focus, composed of mononuclears, is found. The interlobular fibrous connective tissue is somewhat increased; otherwise marked changes are not seen.

In the lungs the capillaries and the veins are much engorged. The alveolar spaces contain much granular detritus, many desquamated endothelial cells and considerable numbers of large bacilli taking Gram's stain. These organisms are also found in large numbers in the liver and the kidneys. No plague bacilli are encountered in the sections.

CASE No. 3. LEFT INGUINAL BUBO.

[Necropsy Protocol No. 940. S. Y. S., male Chinese, 25 years old, from 70 Santo Cristo, Binondo. Ill six days. Died April 14, 1904, at 5.30 o'clock a.m. Post-mortem examination six hours after death.]

The body of a male Chinese, about 35 to 45¹ years old. developed muscles and skeleton. Rigor mortis well marked. mortem lividity of a dark port-wine red color is present over the dependent parts of the body, also around the neck, on the sides of the trunk, and over the anterior tibial regions. The integument in general is quite cyanotic. On the left leg over the sharp edges of the tibia, midway between the ankle and the knee, are seen three small oval ulcerations; they have the size of a split pea and are covered with a dry, dark brown crust. Below these shallow ulcers are about a dozen depressed, healed cicatrices of the same size as the ulcerations. The lymph glands below the left ligament of Poupart are swollen and the skin here is covered with tenacious brown ointment. After its removal the integument is found unbroken, but puffed up and cedematous. The round swelling which protrudes over the surrounding skin has the size of a walnut, feels doughy, and is rather firm; but the individual glands are not distinctly palpable. On section of the skin there escapes first a small amount of yellowish, watery fluid, which becomes bloody as soon as the subcutaneous adipose tissue is cut into. The glands of this region have become fused together, and the individual components of the group are indistinguishable. The whole tissue shows an intense hemorrhagic infiltration and well-marked softening. A good deal of dark, bloody fluid can be scraped off from the cut surface and it can be seen that the hemorrhagic infiltration

¹The deceased was evidently much older than the figure given officially in the death certificate.

extends beyond the glands into the periglandular and general subcutaneous connective tissue. The capsules of the glands are indistinguishable.

After opening the abdomen, the left iliac glands are found in the same condition as those in Scarpas' triangle. They form a bubo of the size of a small walnut. However, those higher up are much less affected; they are moderately increased in size and dark purple in color, but there is no marked hemorrhagic infiltration beyond the gland substance. The peritoneum, the pleura, and the pericardium show deeply injected, dilated vessels. The intestinal serosa appears dull and exhibits slight greenish discoloration; shining through it are found hemorrhagic spots in moderate numbers both in the small and in the large intestine. The pericardium contains a normal amount of clear straw-colored fluid. The epicardium shows greatly congested vessels and a number of flat, slightly elevated, vellowish-gray, old, fibrous cicatricial bands. These are found on the right side, over the auricle and the ventricle. The auriculo-ventricular zone exhibits quite a number of small petechiæ from the size of a pin head to that of a millet seed. The right ventricle is much distended and contains a large amount of dark red, fluid blood; the left ventricle is firmly contracted and contains a small, dark red, firm clot. The heart as a whole is moderately hypertrophied, the walls of the left ventricle are thickened, and those of the right rather thin and flabby. The myocardium in general is firm, and on section of a dull, brownish-pink color. The arch of the aorta shows a number of atheromatous patches. Both lungs are quite heavy, their upper lobes being of a slate-grayish-pink color and the lower ones dark bluish-purple. On the cut surface the upper lobes are moist and their vessels discharge a moderate amount of dark blood; the lower ones are brownish-red and their tissues are very rich in dark, fluid blood and very poor in air. The small bronchi of the lower lobes contain a bloody, foamy, fluid; slightly blood-tinged, foamy, viscous fluid is also found in the trachea. The mucosa of the bronchial tree, of the trachea, and of the larvnx is slightly swollen and much injected. The bronchial glands are not enlarged; however, they are very dark in color and much congested. The spleen is much enlarged, its diameters being 24 by 12 by 5 centimeters. All the hilus vessels are much enlarged. The capsule is smooth and transparent everywhere, except in one place at the

upper convex surface, where it is somwhat thickened, though not elevated, and opaque and dull gravish-white in appearance. The cut surface of the organ is dark brownish-red; the trabeculæ can be seen very well and are distinctly thickened; the pulp is moderately soft; and a moderate amount of juice can be scraped off the cut surface. The Malpighian corpuscles are not distinguishable. On the whole the consistency of the spleen is rather increased than decreased. Its weight is 675 grams. The kidneys are normal in size, much congested, and the capsules are rather dull and not very translucent. The outer surface is dark purplish-blue. Here and there a gravish-white mottling is seen. The capsules peel off easily. On the cut surface the vessels appear injected, the tubules decidedly grayish-yellow. The glomeruli are not very distinct, and the pyramids are dark pinkish-purple in appearance. The relation of the cortex to the medulla is normal. The mucosa of the pelvis is smooth and much injected, so that the small vessels are distinctly visible. The suprarenals are normal in size, moderately injected, vellowish-brown in color, and somewhat softened. The liver is of normal size, the capsule very tense, shining, and transparent. The external color is bluish-purple. The cut surface discharges a rather moderate amount of dark, fluid blood. The liver lobules are distinct and of a dark brownish-yellow color. The gall bladder is distended with very dark, turbid, pitchy bile. The mucous membrane is swollen. There are no stones. The ducts are normal. The serosa of the stomach and intestines is injected, that of the latter being of a dull appearance, as before described. The dark spots seen through the intestinal serosa correspond to hemorrhagic spots of the mucosa. The gastric mucosa shows numerous small, punctiform petechiæ on a dirty gravish background. The pancreas, the prostrate, and the bladder show no particular changes.

Anatomic diagnosis.—Hypertrophy and hemorrhagic inflammation of the left inguinal and iliac glands; passive congestion and parenchymatous degeneration of the kidneys; congestion and cedema of the lungs; multiple subserous and submucous hemorrhages; moderate hypertrophy of the heart; old epicardial cicatrices; atheroma of the aorta; splenomegaly. Bubonic plague.

Smears made from various organs show the following: From the primary bubo, quite a number of very poorly staining oval or round bacilli. Only a very small peripheral rim has taken the dye; the center is entirely unstained. These bacilli appear as empty shells.

Another bacterium present is a long, slender, well-stained bacillus. The juice from the spleen contains a moderate number of plague bacilli; that from the liver and the kidneys a few only; none at all are found in the smear from the heart's blood. Cultures inoculated from the inguinal glands and from the spleen developed plague bacilli; a tube from the liver remained sterile.

Microscopic examination.—The glands of the inguinal region show a marked increase of connective tissue and thickening of the walls of the vessels at the hilum. While some portions still exhibit a fairly normal lymphoid tissue others are thoroughly infiltrated with extravasated blood. The loose, areolar, pericapsular connective tissue is infiltrated with mononuclear and polynuclear inflammatory cells. Innumerable plague bacilli are diffused throughout the gland and penetrate into the periglandular connective tissue. Here and there a moderate amount of fibrin is seen, particularly around some small vessels, but hyaline thrombi obliterating the vascular lumina are not visible. The capsule of the spleen is not thickened, but the trabeculæ are broad. The Malpighian bodies are quite small, not sharply defined, and shade off gradually into the surrounding tissue. The fibrillar connective tissue of these follicles is increased. The pulp spaces are fairly well recognizable and are densely filled with red blood corpuscles. while a moderate number of polynuclears, some typical plasma cells, and large mononuclear cells are also present. The last, which appear to be proliferated endothelia of the pulp spaces, have a large vesicular round or oval nucleus, not very rich in chromatin, with generally one or more distinct nucleoli. A moderate number of plague bacilli is found in the spleen sections. The kidneys show both large and small vessels to be greatly engorged. There is extensive cloudy swelling of the epithelium of the uriniferous tubules, and a moderate amount of granular material is scattered through the latter. The liver exhibits engorged capillaries and a very moderate degree of fatty degeneration of parenchyma cells. Very small interlobular inflammatory foci are seen in a few places. The pulmonary tissue shows an enormous engorgement of the blood vessels. Most of the alveoli are open and empty, but a moderate number contain red blood corpuscles, or more or less granular material, in which are embedded leucocytes and shreds of fibrin, the latter forming a network like that occurring in fibrinous pneumonia. Plague bacilli are not found in such small

incomplete areas of consolidation. The stomach shows engorgement of the veins of the submucosa. The congestion is continued into the small veins and capillaries of the mucosa. Blood is found extravasated between the glands and upon the surface of the latter. The cells lining the peptic glands show evidence of nutritive disturbance. Here and there one sees karyokinetic figures, while a number of the sustentacular central cells show two or more nuclei. Plague bacilli are not seen in the mucosa.

CASE No. 4. RIGHT INGUINAL BUBO.

[Necropsy Protocol No. 932. F. H., young male Filipino, from 20 Alma Street, Tondo. Died March 20, 1904, at 2 o'clock p. m. Post-mortem examination made March 21, at 10 o'clock a. m., twenty hours after death.]

Body of an unusually strong young native, of 20 to 25 years of age. The right heel shows a large, open, ulcerated surface about the size of the palm of the hand, dark purplish in color, covered with a dirty greenish fibro-purulent secretion. The entire anterior surface of both legs shows a scaly vesicular eruption. Post-mortem lividity is well marked all over the body. The skin in general is markedly cyanotic. Post-mortem rigidity has almost disappeared. Putrefaction is well advanced.

On opening the abdomen a great deal of ill-smelling gas escapes. The superficial blood vessels discharge a moderate amount of dark, fluid blood. The serous membranes are quite dull, their vessels are markedly injected. Heart: The left ventricle is well contracted. the right ventricle dilated. The visceral pericardium is strongly injected and shows a few small hemorrhagic areas. The myocardium is pinkish in color, and fairly firm in consistency. Valves normal. Endocardium smooth. The large vessels are normal. Lungs: Slightly adherent to the pleura costalis. Extensively adherent to the upper surface of the diaphragm. The upper lobes are pinkish in color and contain a good deal of air; the lower lobes are highly congested and of a dark purplish color; they contain but little air, are very ædematous and full of dark fluid blood. The mucous membrane of the bronchi and of the trachea is swollen and congested; the air tubes contain a moderate amount of frothy, viscid mucus. Larynx likewise congested. Spleen: Normal in size; the capsule is smooth and slightly wrinkled and the outer surface is dark purple in color. The cut surface is dark brown, the pulp is soft. A good deal of dark-brown juice can be scraped off the surface. The Malpighian bodies are not distinctly visible.

The trabeculæ are fairly well marked. Kidneys: Capsules smooth, surface purplish in color. After removal of the capsules, which peel off easily, the glomeruli stand out as highly injected points, surrounded by a gravish white tissue. On section the glomeruli, the vessels, and the pyramids appear highly congested. The tubules are gravish white. The pelves are smooth and much congested. The surparenals are enlarged, soft, edematous, and congested. Brownish purple in color. Liver: The liver is somewhat swollen, capsules smooth and transparent. Outer surface purplish gray. The cut surface is purplish brown. The vessels discharge a good deal of fluid blood. Boundaries of liver lobules distinct. The gall bladder is of a dark grayish purple color. Its mucous membrane is swollen and congested. The viscus contains a large amount of dark greenish turbid bile. No stones. Stomach and intestines: The serosa is rather dull, showing injected vessels. A number of small hemorrhagic spots are seen on the serosa of the small intestines. The mucous membrane of the stomach is gravish-white and shows a number of small hemorrhagic spots. It is covered with dirty gray, tenacious mucus. The small intestine likewise shows some small hemorrhagic spots in its mucosa. Lymph glands: The inguinal glands of both sides are much swollen, rather soft, edematous, congested, and purplish in color. These changes are more marked on the right side than on the left. The mesenteric lymph glands show similar changes, though to a less degree. cervical glands show only a very moderate amount of enlargement and congestion.

Anatomical diagnosis.—Large granulating ulcer on the right heel. Congestion and cedema of the lungs. Passive congestion of the liver and kidneys. Parenthymatous degeneration of the kidneys. Œdema, general hypertrophy, and congestion of the lymph nodes, particularly of those of the right inguinal region. Multiple subserous and submucous hemorrhages. Bubonic plague.

Smears are made from the heart's blood, spleen, liver, and left inguinal glands. The smears from the heart's blood show only a few pest bacilli, while those from the spleen and glands show a considerable number. Culture tubes are inoculated from the heart's blood, spleen, and liver. The tube from the spleen developed a pure culture of plague bacilli; those from the liver and the heart's blood showed a mixed culture of plague bacilli and staphylococcus pyogenes albus.

Microscopic examination.—The inguinal lymph glands show an ædematous infiltration and great dilatation and congestion of blood vessels. The perivascular connective tissue at the hilum is much increased. An area of extensive blood extravasation is found near the capsule. The majority of the parenchyma cells of the gland are small mononuclear and ordinary polynuclear cells; plasma cells are fairly numerous, and plasma mast cells are plentiful. Here and there are seen globular, homogeneous masses of a diameter of 20 to 30 μ and more which have a strong affinity for eosin. Neither the blood vessels nor the other parts of the tissues show any fibrin. Plague bacilli are only sparingly seen in sections of the glands. Sections of splenic tissue show mostly ill-defined Malpighian corpuscles, obliterated pulp spaces, and an overcrowding of the latter with red blood corpuscles. As in the lymph gland tissue, plasma cells and plasma mast cells are numerous. Bacilli are found in small numbers only. The renal tissue shows greatly dilated and engorged vessels, very small areas of blood extravasation between the tubules, an occasional thickening of Bowman's capsule, and most extensive cloudy swelling of the tubular epithelium. While the latter is still found intact here and there, most tubules, both convoluted and straight, are lined by greatly swollen, irregular, hazy cells in which a nucleus is not seen, or if seen at all, it is very poorly stained.

In the pulmonary tissue one sees enormously engorged interalveolar capillaries and air spaces partly filled with desquamatedepithelia and erythrocytes. No fibrin is found in the alveoli.

CASE No. 5. RIGHT INGUINAL BUBO.

[Necropsy Protocol No. 977. O. C., Chinese, 25 years, male, from 214 San Jacinto Street. Died after an illness of two days on May 25, 1904, 9.15 p. m. Postmortem examination fifteen hours after death.]

Body of a young male Chinese about 25 years old; rather slender, but well developed. Post-mortem rigidity moderately well marked. The skin as a whole is quite cyanotic, and the post-mortem lividity, which has extended well to the anterior surfaces, is marked. A small amount of dirty-brown foamy fluid exudes from the nares. No wounds or ulcerations are to be found anywhere on the integument. The right inguinal region is somewhat swollen, but the swelling is not high; it is not well defined but shades off gradually into the surrounding tissue. The swollen area is markedly cyanotic and cedematous, the skin here pits on pressure. On section the

subcutaneous connective tissue discharges a serous, slightly yellowish fluid, and on further dissecting into the tissues an extensive hemorrhagic infiltration is encountered. This hemorrhagic infiltration surrounds ill-defined, swollen, soft, and hemorrhagic glands. The bloody extravasation extends from Scarpa's triangle through the inguinal canal, into the pelvis along the iliac glands, thence into the abdominal cavity up to the region of the kidney. lymph glands along this course are swollen, much softened, and hemorrhagic. The same pathologic changes, though to a lesser degree, are shown by the mesenteric, the peritoneal, the mediastinal, and the bronchial glands. The latter are more markedly enlarged, softened, and hemorrhagic than any other glands mentioned except those of the chain beginning with the right inguinal glands. The superficial lymph glands, aside from the right inguinal, are moderately swollen, softened, and congested. Subserous hemorrhagic spots are found on the epicardium, on the pleuræ, on the external surfaces of the stomach, the small intestines, the kidneys, and on the capsule of the liver at the insertion of the suspensory ligament. The pericardium is smooth, much injected, and contains a moderate amount of fluid. The heart shows a number of subepicardial hemorrhages, varying in size from a small point to irregular spots several millimeters in diameter. These petechiæ and ecchymoses are found on both ventricular surfaces and on the sulcus. The myocardium is of fair consistency. No further dissection of the heart is made, since it is to be preserved as a museum specimen. The lungs are well expanded, their pleuræ smooth, nonadherent, and dark purplish-blue, with a moderate number of small hemorrhagic spots and with some small elevated emphysematous areas on the lower lobes. On section the pulmonary tissue is of dark purplishbrown color, containing very much dark blood and foamy, aqueous fluid. The lungs on the whole are heavy and contain but little air. The bronchial, tracheal, and larvngeal mucosa is somewhat swollen and greatly congested. The epiglottis is of a dark purplishblue color. The spleen is normal in size, the capsule slightly wrinkled, transparent, and bluish-gray. The organ as a whole is fairly firm. The cut surface is reddish-brown and granular. The amount of juice which can be scraped off the surface is quite moderate. The trabeculæ and corpuscles are distinct.1 The kidnevs

¹ Many plague bacilli were found in the smears from the spleen; yet it was neither enlarged nor softened.

are much congested and very soft. Externally they are dark bluishgray, with some subcapsular petechiæ and ecchymoses. On section the vessels are greatly engorged, the tubules grayish-white, and the surface as a whole dull. The mucosa of the pelvis and bladder is greatly congested. The mucosa of the ureters and the bladder is moderately congested, but shows no hemorrhages. The suprarenals are large, swollen, soft, and dark yellowish-brown. The liver is large, its margins rounded, its capsule thin and transparent, and its external color a yellowish-bluish-purple, alternating with decidedly gravish-yellow areas. Along the insertion of the suspensory ligament are seen numerous subcapsular hemorrhages, varying in size from a mere point to a diameter of 5 to 7 millimeters. The organ is of much increased consistency, and the cut surface is brownishvellow in color. The gall bladder is distended with dark vellowishgreen bile. The serosa of the stomach and intestines shows a number of hemorrhagic spots. The gastric mucosa is studded with small hemorrhagic areas and so is that of the duodenum, though to a lesser extent. The lymph follicles of the intestines are swollen.

Anatomic diagnosis—Congestion of the lungs; congestion and parenchymatous degeneration of the kidneys; fatty degeneration of the liver; multiple subserous and submucous hemorrhages; multiple hemorrhagic lymphadenitis. Bubonic plague.

Smears from the right inguinal glands show innumerable typical plague bacilli; those from the spleen show numerous pest organisms. Culture tubes inoculated from the inguinal glands and the spleen developed a typical growth.

Microscopic examination.—The inguinal glands show an enormous dilatation and engorgement of the blood vessels, a moderate degree of free blood extravasation, and marked cedema. A number of the smaller blood vessels are completely obliterated by hyaline thrombi. Other larger vessels show closely packed blood corpuscles and a network of fibrin between the corpuscles. The number of leucocytes in the engorged blood vessels, including those which show a network of fibrin, is very moderate. If at all increased over the normal, they are not very greatly so. Any damage to the vessel walls, which are much dilated, is not demonstrable, and the vascular endothelium appears intact. The cellular elements of the gland are the same as in the other cases described above. Plague bacilli are very numerous. However, they do not form dense, almost solid masses, but infiltrate the intercellular spaces

and surround, as it were, each individual cell. Spleen: The boundaries of some of the Malpighian corpuscles are distinct and sharply cut: others show an indistinct limitation, because the small mononuclear cells forming the follicles are densely infiltrating the neighboring tissues. The pulp spaces are quite indistinct, because they are crowed with cells. Most of these are leucocytes, but in some places red blood corpuscles predominate. All through the pulp spaces of the splenic tissue a fibrin network can be seen. No fibrin, however, is found in the vessels and the network has, of course, no intravascular connection. Here and there one can see the fibrin threads take their origin from leucocytes. Plague bacilli are present in large numbers. Kidneys: The glomeruli do not show any marked changes, but the capillaries of the tufts are greatly engorged with blood. In general all renal vessels, particularly the capillaries and the smaller veins, are much engorged. In a few places, near the capsule, smalls areas of blood extravasation are encountered; however, none are found at a distance from the surface. The epithelium of the convoluted tubules shows considerable cloudy swelling and also more profound degeneration, with complete loss of nuclei. The tubular lumina are generally filled with more or less granular detritus. Few, and not greatly advanced, changes are seen in the straight tubules. No bacilli are seen in sections from the kidneys. All parenchyma cells of the liver are in an advanced stage of fatty degeneration and their nuclei are either poorly or not at all stained. Aside from this degeneration the hepatic tissue shows no marked changes. Sections from the lungs present greatly engorged capillaries; the alveoli are partly filled with desquamated epithelia, red blood corpuscles, and a granular detritus. Plague bacilli are not found. In the gastric mucosa the interglandular capillaries are much enlarged and free blood is found between the glands up to the very uppermost strata. However, no blood is seen on the free surface of the mucosa.

CASE No. 6. RIGHT INGUINAL BUBO.

[Necropsy Protocol No. 998. Post-mortem examination performed on July 3, 1904, twelve to eighteen hours after death, upon the body of V. D., from 17 Azcarraga Street, Tondo; a male Filipino 17 years old.]

Post-mortem rigidity is not well marked; it has evidently begun to disappear. Post-mortem lividity is noticeable over dependent parts of the body. The integument, particularly around the chest, the neck, and the face is quite cyanotic. The right inguinal glands, particularly the lowermost ones, show considerable swelling. The latter are enlarged to the size of a walnut. The skin is unbroken. The region is quite firm and hard, somewhat ædematous, but not fluctuating. The boundaries of the individual glands can not be well differentiated and the swelling shades off gradually into the surrounding tissues. A chain of swollen indurated glands can be felt along the spermatic canal. On opening the body, the abdominal cavity shows a moderate amount of slightly turbid fluid. The serosa is dull. The intestinal serosa is deeply injected, and here and there small hemorrhagic spots are seen. The general color of the intestinal serosa is varied by some greenish discoloration due to putrefactive changes. On the right side all the iliac glands are swollen, much congested, and more or less hemorrhagic on section. In fact, the whole chain of glands from those in Scarpas' triangle up to the retroperitoneal ones, and as far as the kidneys, is in this condition. The hemorrhagic infiltration is most marked in the inguinal glands. There it extends into the periglandular connective tissue, the individual glands have become confluent, and their capsules and their finer structure have become indistinct. The heart shows a larger number of irregular subepicardial hemorrhages, measuring from 1 to 5 millimeters in diameter. They are situated over the left ventricle. The myocardium is fairly firm, pinkish, with a slightly yellowish tint. Otherwise normal. The lungs are very slightly adherent by some few thin adhesions. The upper lobes contain a fair amount of air and are pinkish-purple in color. The lower lobes are heavy, congested, edematous, and purplishblue in color. A few subpleural hemorrhagic spots are seen on the lower lobes. Bronchi, trachea, larynx show an injected mucosa. The papilæ circumvallatæ of the tongue are much swollen. The spleen is 15 by 12.5 by 6 centimeters. It weighs 645 grams. Capsules smooth, transparent, steel-grayish-blue; consistency fairly firm. Cut surface brownish red. Malpighian bodies not very distinct, trabeculæ distinct; cut surface slightly granular, amount of juice which can be scraped off, moderate. Kidneys normal in size, soft; capsules smooth, peel off easily. Pinkish purple showing a few small subcapsular hemorrhages. Cut surface, vessels injected, tubules vellowish gray, dull. Pelves smooth. The lower third of the left ureter is dilated to three times its normal diameter. The bladder contains several ounces of turbid urine. On the right side posteriorly below the apex, there is seen a flat elevation about

the size of a split bean. There the tissue is purplish-pink and the swelling appears somewhat like an enlarged lymph gland. On section this part seems somewhat tubercular, and one of the nodules projects into the vesical mucosa. Otherwise the mucosa is smooth, moderately congested. Prostate normal. Suprarenals swollen, soft, yellowish-brown. Liver rather small. Capsule smooth, transparent, much grayish-yellow mottling alternating with a pinkish-purple. On section, veins moderately filled, cut surface slightly granular, boundaries of acini distinct; color ocher-light brown. Gall bladder normal. Gastric duodenal and general intestinal serosa and mucosa injected. Lymph follicles of small and large intestines somewhat swollen. Mesenteric, retroperitoneal, and other lymph glands swollen and congested.

Anatomical diagnosis.—Congestion of the lungs; congestion and parenchymatous degeneration of the kidneys, splenomegaly, interstitial hepatitis with fatty degeneration, hemorrhagic lymphadenitis of the right inguinal glands. General hypertrophy and congestion of lymph glands. Subserous and submucous hemorrhages. Bubonic plague.

Smears from the right inguinal glands show numerous, those from the spleen a moderate number of plague bacilli. No Donovan-Leishman bodies found in the splenic juice. The liver smears exhibit a very few plague bacilli. The culture tubes inoculated from the right inguinal glands and from the spleen developed typical plague colonies.

Microscopic examination.—Sections from the hemorrhagic areas of the right inguinal glands show an abundant infiltration with plague bacilli, particularly well pronounced in some peripheral areas. Bacilli, while numerous, are not present anywhere to such an extent as to form solid, clumped masses. Even where they are most abundant there are a few cells left between them. In general, the histologic elements can be much better studied in sections of this case than in those from glands which are simply choked by solid colonies of the plague organism. The general characteristics of the gland sections are a universal hemorrhagic infiltration with marked cedema, increased diastases between the original parenchyma cells, swelling of the fibrillar connective tissue reticulum, the presence of numerous leucocytic elements, and geat dilatation and engorgement of the vessels. Both in transverse and in longitudinal sections of small veins, considerable damage to the vessel wall is

The cells forming these walls show coagulation necrosis, manifest. hyaline degeneration, and more or less complete loss of their nuclei. The perivascular concentric fibers are loosened and pushed apart from each other by a leucocytic infiltration. The lumina of these very much dilated vessels are crowded with apparently normal red blood corpuscles, and with a slightly increased number of leucocytes. Plague bacilli are not seen in the vessels. The original parenchyma cells, the small mononuclears, show as a rule, a normal nucleus. In some nuclei there is considerable pyknosis, while others are quite poorly stained. Mononuclears, with large hyaline protoplasmic bodies, are only rarely seen, plasma mast cells likewise are scanty. The very numerous polynuclears are generally of the ordinary type, a few are typically coarsely granular eosinophilics, while some have an angular vacuolated protoplasm which does not show any granules but stains very deeply with eosin. The infiltrating, extravasated erythrocytes are mostly, to all appearances, normal; however, in some places they have become agglutinated and confluent, almost completely forming homogeneous masses, deeply staining with eosin. No complete hyaline thrombi are found in the vessels, but a few of the latter show an open network of fibrin. Such fibrin reticula are also found here and there free between the cells. In the spleen the pulp spaces show a considerable number of large, proliferated endothelial cells. These appear to be derived from the lymphatic endothelia lining the pulp spaces. They are generally phagocytic cells, containing from two to twelve and more engulfed mononuclears, polynuclears, and erythrocytes. The picture which the pulp, with its many large endothelia, furnishes is much like that seen in typical primary splenomegaly or splenic anæmia, but the generally marked phagocytic character of the large pulp cells is analogous to the condition found in the spleen in typhoid fever. Plague bacilli are found very sparingly in the splenic sections. Kidneys: The epithelial lining of the convoluted, as well as of the straight tubules, is in an advanced state of cloudy swelling and fatty degeneration. Some of the canaliculi contain a moderate amount of granular matter. The glomeruli do not show any marked changes. All of the vessels are much engorged and small subcapsular hemorrhagic areas are encountered. Liver: The veins and capillaries are dilated and they contain much blood. The protoplasm of the parenchyma cells shows fine, dust-like vacuolation; their nuclei are generally well stained. Some of the liver

cells are swollen, hazy in outline, and without nucleus. The interlobular septa contain acute inflammatory foci mostly composed of small mononuclear cells with a few neutrophilic and a still lesser number of eosinophilic polynuclears. The pulmonary sections are characterized by enormously engorged capillaries with some blood extravasated into the alveoli. In some places an increase of the interalveolar connective tissue is noticeable. Otherwise there are no important changes. The microscopic examination of the nodule found in the bladder shows it to have the structure of a subacute inflammatory focus, composed of connective tissue fibers, small mononuclears, and some polynuclear eosinophilics. Quite a number of bacilli are seen in this area. They do not retain Gram's stain; however, they appear smaller and more delicate than plague bacillus, and they stain more uniformly than the latter.

CASE No. 7. RIGHT INGUINAL BUBO.

[Necropsy Protocol No. 1000. July 5, 1904. Post-mortem examination, thirtynine hours after death, on the body of G. A., from No. 43 Valderana Street, San Nicolas; a Filipino boy about 10 years old.]

Post-mortem rigidity has disappeared and putrefactive changes are well advanced. The skin in the region of the abdomen shows green discoloration, as does also that of the neck. inguinal region exhibits a swelling about the size of a walnut, and here the boundaries of enlarged glands can be made out by palpation. On section, the tissues are found to be ædematous and slightly hemorrhagic. The glands are much enlarged and hemorrhagic; but their capsules can still be distinguished and they are well differentiated from the surrounding tissue. The latter is likewise hemorrhagic, but to a less degree. On the cut surface the glands of the right inguinal region appear mottled; dark-red areas predominate and alternate with gravish-white ones. Considerable turbid and hemorrhagic juice can be scraped off. The glands from Scarpa's triangle, through the abdominal ring, up into the pelvis, are swollen, softened, and hemorrhagic. The right iliac glands as well as the right retroperitoneal ones are in the same condition. The alterations found in the internal organs, not considering putrefactive changes, are as follows: Heart: Two small subepicardial hemorrhages; myocardium, soft, flabby, and pinkish-brown. The lower lobes of the lungs are bluish-purple and quite ædematous, showing a few small subpleural hemorrhages. The spleen is enlarged to twice the size of the adult organ; it is bluish-purple externally,

dark brownish-red on the cut surface, and granular; much juice can be scraped off. The trabeculæ and Malpighian corpuscles are fairly well marked. Kidneys: The right kidney is much congested and dark purplish-blue; the cut surface shows injected vessels and somewhat yellowish tubules. The mucosa of the pelvis is injected and shows several hemorrhagic spots. The left kidney is also much congested, but rather pale vellowish-gray on the cut surface. The tubules are decidedly grayish-yellow and quite dull in appearance. The pelvis is smooth and not markedly congested. The ureters and the bladder are normal. The liver is rather large with rounded margins and much yellowish mottling alternating with bluish-purple areas. The cut surface is reddishbrown and the vessels much injected. The mesenteric, retroperitoneal, bronchial, and other glands are all enlarged, congested, and rather soft and juicy. There is nothing abnormal about the intestines excepting a moderate swelling of the lymph follicles. The serosa and the mucosa of the stomach are much injected; there are no hemorrhages.

Anatomic diagnosis.—Hemorrhagic lymphadenitis of the right inguinal glands; multiple lymphadenitis with great congestion and softening; congestion of the kidneys and parenchymatous nephritis; fatty degeneration of the liver. Bubonic plague.

Smears from the right inguinal glands show numerous plague bacilli, those from the spleen only a moderate number. Tubes inoculated from the glands developed a typical growth.

Microscopic examination.—The right inguinal glands and those of the chain which leads into the right iliac fossa show changes resembling those in the other cases of hemorrhagic plague buboes. The bacilli are present in very large numbers; however, solid masses of them are found only in a limited area. In general, they densely infiltrate the hemorrhagic and edematous tissue. The tissue elements of the infected glands in this case include a very considerable number of mononuclears with a large body of protoplasm. The latter is hyaline and stains rather well with methylene blue. A number of these large mononuclear cells contain bacilli in their protoplasm, and the cells of this type are the only ones which exhibit this phagocytic tendency. That the bacilli are really inside the protoplasm and not on top of it can be seen in very thin sections from edematous areas where isolated cells can be studied. Some of these mononuclears have engulfed other cells.

The other cells in the gland sections are of the usual type—small mononuclears, plasma cells, mast cells, and neutrophilic and eosinophilic polynuclears. A number of vessels contain a network of fibrin. Such reticula are often seen independently of vessels in several parts of the sections. A continuation of the intravascular fibrin network to the extravascular reticula is nowhere demonstrable. The kidneys show profound parenchymatous degeneration with cloudy swelling; hyaline glomerular thrombi are not found. The post-mortem changes in the renal tissues are very advanced, so that the finer histological changes are not well preserved. liver cells are in a condition of cloudy swelling and fatty degeneration with both fine and coarse vacuolation. The interacinous connective tissue is much increased. The septa contain numerous connective tissue fibers and infiltrating inflammatory cells. This change is so pronounced as to be due evidently not merely to plague infection but to some cause acting before the latter occurred. The interlobular inflammatory foci do not show plague bacilli. The capillaries and veins are much engorged. In the spleen the boundaries of the follicles are generally sharp and the Malpighian bodies are well differentiated from the surrounding tissue. pulp spaces, however, are not distinct, since they are densely crowded with cellular elements. The red blood corpuscles predominate in number. The different types of leucocytes include many of the large mononuclear cells with large hyaline protoplasm, staining faintly with methylene blue. In the spleen, as in the lymph glands, some of these large hyaline mononuclears contain plague bacilli in their protoplasm. A few of the phagocytes contain other leucocytes or erythrocytes. Plague bacilli are only sparingly found in the splenic sections.

CASE No. 8. INGUINAL BUBO.

[Necropsy Protocol No. 965. R. F., native, female, 45 years old, from No. 33 Calle Victoria, Intramuros. Died May 7, 1904, at 11.45 p. m., said to have been sick four days. Admitted to San Lazaro Hospital on May 7 at 11.30 p. m., and died fifteen minutes later. Post-mortem examination eleven hours after death.]

The body of a strong, stout woman, about 45 years of age. Postmortem rigidity is well marked; post-mortem lividity is extensive. There are large livid patches on the anterior surface of the thighs and trunk. No petechiæ or ecchymoses are seen. The integument shows several old healed cicatrices, which might have been produced

by a sharp cutting instrument. There are no ulcerations, wounds, or recent cicatrices. None of the superficial glands are palpable, but on being dissected out, the inguinal glands on both sides are found to be much enlarged and congested, but relatively firm and not hemorrhagic. On section, the superficial vessels discharge a moderate amount of dark fluid blood. The serous membranes are shining, very moderately injected, and the serous sacs contain small amounts of clear fluid. The pericardium is smooth and normal. The left ventricle of the heart is well contracted and the right one moderately dilated. The valve openings, the endocardium, etc., are normal. The myocardium is moderately soft, of a reddish-brown color, and not very easily torn. The beginning of the aorta is atheromatous; the coronary vessels are moderately engorged. lungs are slightly adherent in a few places and collapsed. Their pleural surfaces are smooth and even. The lower lobes are uniformly congested; the upper ones are very moderately so and pinkish-gray in color. The former, on section, are found to be engorged with dark fluid blood and to contain little air; the latter have a moderate amount of blood and more air. The bronchi are filled with a foamy, slightly blood tinged, viscid mucus. Their mucosa is somewhat congested, as is also the mucous membrane of the trachea and the larynx. The spleen is very much enlarged, measuring 20.5 by 12 by 7 centimeters and weighing 865 grams. It has retained the general shape of the organ and its capsule as a whole is thickened and nowhere transparent, but rather opaque. The surface is in general grayish-blue with some areas which are grayish-white. A larger area of this type is found in the center of the upper surface; it is slightly raised above the surrounding tissues and quite opaque. The vessels entering at the hilum are much enlarged in caliber, particularly the veins, which are rather thin walled. The organ is quite firm in consistency. On section, the pulp does not protrude over the cut surface, but is even, quite firm, and of reddish-brown color. The trabeculæ are increased in width and stand out prominently. The Malpighian bodies are not distinct. A very moderate amount of juice can be scraped off the cut surface. The kidneys are different in size, the left one being much larger than the right. The weight of the former is 165 grams and that of the latter is 120 grams. Both have smooth capsules and their external surfaces are bluish-pink in color. On section, the vessels are found to be much engorged, the glomeruli

injected, the tubules grayish-white, the pelvis smooth and slightly injected, and the cut surface dull. The liver is very firm in consistency. Its capsule is slightly uneven and finely nodular. The external color is gravish-vellow. The organ is rather small, and the left lobe in particular is flat and atrophic. The measurements are 22 by 12 (high) by 13 (antero-posteriorly), right lobe; the left lobe is only 2-2.5 centimeters thick. The weight is 1,595 grams. There is increased resistance on cutting the organ. color of the cut surface is ochre yellow. The acini are retracted, and the boundaries are well marked by an increase in the interlobular connective tissue. The vessels discharge a moderate amount of blood. The gall bladder is distended and contains a deep goldenvellow turbid bile. Its walls are normal and its mucosa smooth. The ducts are open. The mucosa of the stomach and duodenum are moderately injected, the injection being most marked in the gastric mucosa. There are no petechiæ or ecchymoses. The uterus is small and hard; the uterine mucosa is thin and atrophic. The ovaries are small and nodular. There is no fresh corpus luteum. Otherwise the genital organs are normal. None of the internal lymph glands show marked changes.

Anatomical diagnosis.—Splenomegaly (primary?); perisplenitis; cirrhosis of the liver with moderate fatty degeneration; congestion and parenchymatous degeneration of the kidneys; congestion of both lungs; Banti's disease. Bubonic plague.

After the completion of the post-mortem examination, it was thought that this was not a case of plague but one of some other infection which had taken a speedy fatal termination on account of a complication with splenomelagy and hepatic cirrhosis. However, the examination of the smears made from the inguinal glands revealed the presence of very numerous typical plague bacilli. Few such organisms were found in spreads from the spleen, the liver, and the lungs, while none were found in the heart's blood. Cultures from the spleen and the lungs developed a plague growth. No bacilli grew in the tube inoculated from the heart's blood.

Tissues were taken from both lungs, the liver, the kidneys, the spleen, and the heart, but none had been taken from the glands.

Microscopic examination.—Spleen: The capsule is markedly thickened, consisting of connective tissue fibers, spindle-shaped cells, and occasionally an unstriped muscle fiber. An accumulation of round mononuclear cells exists at the inner surface of the capsule.

The subcapsular lymph sinus is not very distinct, though to some extent it may be recognized. The trabeculæ are thickened; their connective tissue is poor in nucleated cells and consists mostly of wavy fibers. The larger arteries show considerable hypertrophy of the adventitia. The Malpighian bodies can hardly be recognized. The accumulation of lymphoid cells around the terminal arteries forming these bodies has become much rarified, the boundaries of the cells have almost completely disappeared, and they are gradually lost in the surrounding tissue. With a low power no pulp spaces can be distinguished, but with high magnification the original ones can be recognized, at least here and there, as narrow clefts. The predominating cell element in these sections is the mononuclear type, with a vesicular nucleus, a reticular chromatin, one or more nucleoli, and a protoplasmic body, generally of medium and frequently of considerable size. These cells are clearly proliferated lymphatic endothelia derived from those which lined the original normal pulp spaces. Therefore, the great increase in volume of the spleen is to be attributed mainly to an endothelial proliferation, as several writers have previously described in certain cases of primary splenomegaly (among them the author of this report). A considerable number of polynuclear neutrophiles are also found in the pulp spaces, as well as some mononuclear plasma cells. Red blood corpuscles are present to a certain extent, though they are not so numerous as in an equal area of a normal spleen. Some of the larger mononuclear endothelia are phagocytic, containing red or white blood corpuscles, or both. (None were seen which included plague bacilli.) Here and there in the sections one sees dense and more or less irregular masses about 20 to 30 μ or more in diameter, which show a marked affinity for the eosin stain. It was first believed that these were peculiar cells, but it was finally decided that they were composed of deformed, agglutinated red blood corpuscles, sometimes including a mononuclear or polynuclear leucocyte. Plague bacilli generally arranged in small groups were found in the pulp spaces or in the outer parts of the ill-defined follicles. A search for the Donovan-Leishman bodies was negative. Liver: The interlobular hepatic tissue is increased and some of the liver lobules show marked atrophy. The septa between the acini exhibit both old connective tissue fibers without nuclei and nucleated inflammatory cells. Most of the latter are mononuclears of the small lymphoid type. Plasma cells are seen only very rarely, but ordinary polynuclears are fairly numerous. The interlobular inflammatory foci also contain the same type of eosinophilic masses described above as occurring in the much enlarged spleen. The intralobular capillaries are quite distended and show an increased proportion of polynuclear leucocytes. Many of the parenchyma cells are in an advanced stage of fatty infiltration and degeneration. Few plague bacilli are seen in the liver sections. Kidneys: The capsule is somewhat thickened and a few subcapsular inflammatory foci are seen. They are composed of small mononuclear and embryonal connective tissue cells. The epithelia of the uriniferous tubules are in a condition of advanced cloudly swelling or show the vacuolation of fatty degeneration. The lumina of the tubules contain desquamated cells, mere cell shadows, detritus, and granular material. A few hyaline casts are also occasionally seen staining with eosin but not with the fibrin dye. Some of the small veins contain fibrin; none, however, is found in the glomerular capillaries. The pulmonary tissue shows engorged interalveolar capillaries and in some places a broadening of the interalveolar septa. Otherwise there are no marked changes. A very few plague bacilli are found in some of the sections.

CASE No. 9. RIGHT CERVICAL BUBO.

[Necropsy Protocol No. 928. C. S., Filipina, age 5 years, from 170 Estero San Nicolas. Sick five or six days; three days in San Lazaro Hospital. Died March 18, 1904, at 11 o'clock p. m. Post-mortem examination three hours after death.]

The body of a little girl well nourished. Post-mortem rigidity and lividity marked, the latter extending well over the thorax and neck. The integument in general is quite cyanotic. The cervical glands along the right side are swollen, so that the whole mass has the size of a small apple. This region shows a very intense cyanotic condition. The tissues are very cedematous, imparting a doughy, almost fluctuating sensation to the touch. The swelling shades off gradually into the surrounding areas and in fact extends over the whole of the right side of the face. The eyelids of the right orbit are somewhat swollen and cedematous and completely closed. On incising the body, a small amount of fluid blood escapes from the veins. The stomach and the intestines are much distended with gas. The gastric veins are much congested, the injection being particularly noticeable in those along the larger curvature of the stomach. The intestinal serosa likewise shows injection of its

vessels, and there are a number of dark, hemorrhagic spots, particularly on the ilium. The mediastinal, the mesenteric, and in fact all of the glands which can now be inspected, are swollen, highly congested, and dark purplish in color. The large thymus persists; it is of a dark purple color and shows a number of very dark, almost black, hemorrhagic spots. Such hemorrhages are seen also on the visceral layer of the pericardium. The coronary veins and their branches are much injected. The myocardium is fairly firm, well contracted on the left side and dilated on the right, and of a pale pinkish color. The valves are normal. The lungs are generally pinkish-purple in color and the subpleural veins are much injected. The upper lobes contain a fair amount of air, but the lower ones very little and are much darker than the former, a good deal of dark, fluid blood escaping from them on section. The bronchial glands are in the same condition as the others, being swollen, softened, and highly congested. The spleen is much enlarged, its size being more than that of a normal adult's. Externally it is dark purplish-blue. The capsule is shining and the surface slightly wrinkled. It is soft in consistency, and on section is of a brownish-red color and has a very soft pulp. The Malpighian bodies are fairly well marked, but the trabeculæ are not easy to distinguish. A good deal of dark brown juice can be scraped from the surface. The liver is swollen and of a dark purplish color with an occasional alternating gravish-white area. Its margins are rounded. On section the veins are found to be filled with dark fluid blood. The cut surface is pinkish-brown in color, with some grayish-white areas. The gall bladder is deeply injected and studded with hemorrhagic areas of a deep purple, almost black, color. Its walls are much thickened, very cedematous, and almost gelatinous in character. The mucous membrane is swollen and its small vessels are so highly injected that they can be seen with the naked eye as dark red tortuous lines. The kidneys are pinkish-purple in color, with a number of subcapsular, dark, hemorrhagic areas, which vary in size from that of a millet seed to that of a pea. The capsule peels off easily, and on its removal the glomeruli are seen as dark red points surrounded by rather grayish-white areas. The tubules are grayish-white. The mucous membrane of the pelves is smooth, but even here the small vessels are so much injected that they can easily be distinguished with the naked eve. The suprarenals are much swollen, ædematous, and

of a dark purplish-brown color. The stomach contains a few ounces of a dark greenish fluid, in which are seen grayish-white flocculi. The mucous membrane is thrown into well-marked ruge, and is rather pale on the whole, but contains many very small, irregular hemorrhagic spots. The mucous membrane of the duodenum likewise shows numerous small hemorrhagic spots. pancreas is normal in color and slightly softer than usual. cutting into the cervical glands of the right side, the tissues are found so highly edematous that they discharge a considerable amount of clear, slightly vellowish fluid. All the glands of this region are much swollen, almost black in color, and very œdematous. The deepest ones near the angle of the inferior maxilla, the submentals, are the largest of the group, being increased to the size of walnuts. The cervical glands of the left side likewise show a good deal of congestion and œdema; however, they are small when compared with those of the right side. The inguinal glands of both sides are swollen, congested, and ædematous. In fact, all of the glands of the body which are examined during the post-mortem are in this condition. The trachea, larynx, and esophagus are highly congested. On inspection of the mouth it is found that the front teeth are small, irregularly set, and partly decayed. right side of the soft palate is completely perforated by an ulcer which has destroyed most of the tissues forming the pillars of the fauces. The margins of the ulcer, which is about 2 centimeters in diameter, are irregular and somewhat raised. Contracted cicatricial tissue is found in the neighborhood of the ulcerations.

Anatomic diagnosis.—Perforating ulcer on the right side of the soft palate; general hypertrophy; congestion and hemorrhagic cedema of the general lymph glands of the body; cedema and congestion of the lungs; congestion and parenchymatous degeneration of the kidneys; cedema of the gall bladder; multiple subserous and submucous hemorrhages; syphilis hereditaria tarda. Bubonic plague.

Smears from the different organs show the following: Those from the lungs a moderate number of typical plague bacilli; from the liver a somewhat larger one; and from the spleen an enormous number, these last showing the bipolar staining in a typical manner. In the preparations the common involution forms of the bacilli are also to be observed. Smears from the cervical lymph nodes show a large number of bacilli.

Agar culture tubes inoculated during the post-mortem examination from the heart's blood, the liver, and the spleen developed typical plague cultures.

Microscopic examination.—Sections from the submental glands of the right side show much enlarged and congested vessels, general cedema, areas of blood extravasation, varying from the accumulation of a few red blood corpuscles to extensive and widely spread hemorrhagic areas. The finer structures of the gland are almost completely obliterated and the cortex and medulla are indistinguishable. The gland cells are mostly of the usual mononuclear type. Here and there plasma cells are found. Eosinophilic polynuclears are very scanty. The vessel walls show little or no change, appearing fairly normal even in the middle of areas of extravasated blood. Hyaline thrombi are not found in the glandular vessels. The whole of the tissue is densely infiltrated with innumerable plague bacilli, which are characteristic in shape and show polar staining. The capsule and the surrounding areolar tissue are ædematous and infiltrated with blood. The whole periglandular tissue is completely permeated by a cellular exudate. At the hilum the vessels are enormously enlarged and the perivascular tissue is increased and quite cedematous, so that the individual connective fiber tissues are separated by wide diastases. In spite of these profound changes, there is comparatively little advanced coagulation necrosis and areas where the nuclei have lost their staining properties are few and far between. In the spleen the Malpighian corpuscles are generally distinct in outline, although in some of them the boundaries have become indefinite. Here and there one of the corpuscles shows a proliferating center with large mononuclears, but without mitotic figures. The pulp spaces are not distinct because of dense crowding with erythrocytes and leucocytes. Among the latter there are seen small mononuclears, ordinary polynuclears, and a considerable number of eosinophilic polynuclears. There are also found fairly numerous large mononuclears with large, round, vesicular nuclei and a protoplasm which stains to some extent with methylene blue, though not as markedly as the plasma cells. Some of these cells show two nuclei; they are very probably proliferated endothelial cells. None of them in this case show phagocytic properties. Numerous plague bacilli are found in the pulp spaces, but very few, if any, in the corpuscles. The thymus shows the normal lymphoid tissue with included con-

centric bodies of Hassall; no atrophic changes are yet noticeable. A very prominent feature of sections of this gland is the presence of very numerous plasma mast cells with large, eccentrically situated vesicular nuclei and many coarse basophilic granules. The gland tissue proper does not contain any extensive areas of extravasated blood. However, the capsule and the periglandular loose areolar tissue show considerable blood extravasation. Only very few bacilli can be found in these sections. The renal tissue shows a moderate degree of cloudy swelling. Many of its tubules are more or less completely filled with a granular material, some of them containing hvaline casts. A moderate number of glomerular vessels show hvaline (fibrin) thrombi. Nowhere is this thrombosis very extensive or at all complete; it affects only a minor part of the vessels of one glomerulus. The renal vessels in general are very much congested, and a very few small areas of blood extravasation are found. The few bacilli which are visible are found in connective tissue—lymph clefts or in the capsular space of a glomerulus, but not inside of blood vessels. The liver shows a few small interlobular inflammatory foci and a moderate degree of fatty degeneration of the parenchyma cells. The interlobular capillaries are much distended with blood. No areas of free blood extravasation are seen. The mucosa of the gall bladder is practically normal, although the walls of the organ are intensely edematous. The cedema and extravasated blood have infiltrated the connective tissue so extensively that the fibers form a loose open network, the meshes of which are filled with numerous pale, red blood corpuscles. From the erythrocytes the infiltrating fluid has extracted almost all of the hemoglobin, so that they do not stain well with eosin. The veins in the mucosa of the stomach are much dilated and are frequently surrounded by small periphlebitic areas of cell infiltration. The congestion of the veins of the submucosa is continued into the interglandular capillaries, from which blood extravasation has infiltrated the tissue. Some of the extravasated blood is found between the fixed cells of the mucosa, some of it being deposited free on the surface. The picture seen in these sections is very much like that found in a menstruating endometrium. Sections from the lungs, the heart, and the pancreas do not show any pronounced changes. The hemorrhagic areas in sections from the different organs were carefully examined for plague bacilli, but none were found. Hence the changes must be looked upon as due to toxic

effects and not directly to the presence of the bacilli. In fact, even in the large hemorrhagic areas of the lymph glands bacilli were practically absent, though they abounded in the neighboring ones formed by the fixed tissue elements. Stress is also to be laid upon the fact that these organisms, as a rule, were not found in the blood vessels of the sections examined. The perforating ulcer of the soft palate, surrounded by cicatricial tissue, in connection with the defective incisors, suggested the diagnosis of syphilis hereditaria tarda. It appears that the chronic syphilitic perforating ulcer became the portal of entrance for the plague virus.

INSECTS AS PLAGUE CARRIERS.

Among the plague cases investigated there is one in which it appears probable that the infecting bacilli were carried into the body of the patient—a child—by *Pediculi capitis*. It is advisedly stated that this mode of infection appears very probable, because it is naturally impossible beyond doubt to be certain as to such an occurrence. Only experimental trials on human beings could firmly establish the possibility of this mode of transmission; but such tests with plague bacilli are of course inadmissible.

The question whether insects, and particularly parasitic ones, play a more or less important role in the spread of plague is still far from being definitely settled, as the views of investigators and writers upon this subject are diverse.

Among the modern workers on plague who first gave their attention to insects as plague carriers is Hankin, who reports on a long series of researches on the relation of ants having fed upon rats dead of plague to the possible spread of the disease. He found that ants neither die from plague nor retain the infection for any length of time. By preparing extracts of ants shortly after they had fed upon rats dead of plague, Hankin obtained a fluid which, when injected into rats and mice, would kill and produce typical plague lesions in these animals.

Ogata, in studying a plague epidemic in Formosa, took seven fleas from a rat dead of plague, crushed them between sterile slides, and injected the material so obtained into two mice, one of which died, while the other remained well.

La Bonadiere and Xanthopulides, as they maintain, by cultural methods demonstrated the presence of plague bacilli in a mosquito (?) (moustique) which had bitten a patient sick with plague.

Simond is inclined to attribute much importance to rat fleas as plague carriers. In fleas taken from rats infected with the disease he found bacilli similar morphologically to those of plague. He inoculated three

mice with material obtained from such fleas, but only one of them died, the other two survived.

Loir goes so far as to see the principal intermediary carrier of plague between rat and man in the flea. But, as Galli-Valerio has pointed out, Loir has really no valid experiments to support his claim, which he sees fit to bring forward as a demonstrated fact.

Kolle has systematically attempted to infect healthy rats from those sick with or dead of plague through the agency of fleas. However, he has never succeeded in transmitting plague in this manner, though he was able to demonstrate that fleas had really traveled from the sick rats to the healthy ones. From his observations on rats he thinks that the disease spreads among these animals because the living eat the cadavers of the dead of their own kind and not because of fleas which pass from the plague-sick to the healthy individuals. The primary bubo in rats dead of plague is generally found in the submaxillary region, which points to an infection through a slight lesion of the buccal mucosa. Kolle, in speaking on this subject, very properly remarks: "It is clear that plague bacilli will enter the body of parasitic insects which suck the blood of animals sick with the plague. It has, however, not yet been shown beyond doubt that the bite of such parasites will infect other healthy animals."

Nuttall made experiments to clear up the question as to what part insects play in the spread of plague. He demonstrated that when flies had been fed on material containing plague bacilli they still harbored the virulent organisms twenty-four and even forty-eight hours after the last feeding. According to this author, the danger of a spread of the disease through bedbugs is negligible. The evidence in favor of the "flea hypothesis" as far as the spread of plague is concerned, Nuttall considers worthless and unable to withstand scientific criticism. He states that all of his attempts to infect rats or mice through the bite of freshly infected fleas have proved futile.

Galli-Valerio severely and justly criticises the evidence so far brought forward to show that rat fleas play the most important part in the dissemination of plague among the human inhabitants of a district infected with this disease. He particularly shows that most of those who, on very insufficient evidence, assumed that fleas from rats spread the plague among human beings, have not even ascertained whether the species of fleas infesting the rats will bite man. Galli-Valerio found Typhlopsylla musculi and Pulex fasciatus on European rats and mice under normal conditions. He allowed himself to be invaded by fasting individuals of these two flea species, but neither of them bit him. By this investigator Pulex serraticeps, the flea infesting dogs and cats and which occasionally bites man, was not found on rats.

Tidswell, who made his observations during the latest plague epidemic at Sydney, reports that he collected one hundred fleas from rats, of which ten were identified as *Pulex fasciatus*, eight as *Typhlopsylla musculi*, one as *Pulex serraticeps*, and eighty-one as *Pulex pallidus*. The last one, it appears, has not previously been mentioned as occurring on ordinary

rats; its stated hosts are, according to Thompson, Mus albipes of Socotra and Herpestes ichneumon of Egypt. This species, the author states, bit human beings in laboratory trials, as did also Pulex fasciatus on one occasion. Typhlopsylla musculi did not bite.

Thompson, who, during the Sydney epidemic, observed blebs which he considered to be produced by fleas and to be the place of entrance of the plague virus, concludes that the transmission of plague from rats and mice through the intermediation of fleas must be frequent.

Zirolia believes that plague can easily be spread by fleas. He observed Pulex irritans and P. serraticeps, after they had been fasting for some time, to suck blood from a plague-infected mouse, and he found living, virulent plague bacilli in the bodies of these fleas seven to eight days thereafter. Zirolia also says that the feces of fleas from plague-infected animals contain virulent bacilli, and that in the bodies of the dead fleas these parasites survive for a long time.

Maxwell, from his observations made at Changpoo, China, states that he is coming more and more to doubt the rat-flea theory. "I can not see," he says, "how we can escape plague. I must have been bitten, in spite of flea powder, many times off plague patients and so must my students. The Chinese, especially the women, catch the fleas and kill them with their teeth. If they catch fleas with plague bacilli in them, how do they escape?"

The Indian Plague Commission, which studied plague in all of its phases in India, has also looked into the question of insects as carriers of the disease, and in its report states that Simond's endeavors to establish the proposition that suctorial insects play an important part in the transfer of plague from sick to healthy animals is so weak as hardly to deserve consideration. The experience of plague hospitals in India, and especially that of the Arthur Road Hospital at Bombay, seems to indicate very clearly that suctorial insects do not come into consideration in connection with the spread of plague. The staff and attendants in the Arthur Road Hospital (where thousands of plague cases are treated) were continually bitten by insects, especially mosquitoes, and yet no cases of transfer of the infection from the sick to the healthy came under observation.

The commission also states on reviewing all the facts which have come to its knowledge, that it has little reason to suppose that ordinary, casual contact with plague-infected rats, dead or alive, is especially liable to convey the disease. On the other hand, examples are known of cases where the bite of plague-infected rats and other animals has conveyed the disease.

¹ Galli-Valerio, trying to invalidate Tidswell's observations, says that this author, like others, failed to transmit plague from rat to rat through the agency of fleas and proposes the question: "If the transmission is so difficult from rat to rat, why, on the other hand, should it be so frequent from rats and mice to man, who is not as a general rule attacked by mouse and rat fleas?"

However, the report of the commission does contain some information which suggests that pediculi may be factors in the spread of plague. According to the Bombay statistics of the plague epidemic of 1896, the rate of mortality per 1,000 individuals among the Jains of that city as compared with that among other castes is surprisingly large, and it is believed to be due to the fact that animal life is sacred among the Jains. "They will not," the report states, "sweep their staircases, or sweep their sleeping rooms, or their cooking rooms very often, lest they should destroy some animal life, so scrupulous are they. * * * Not that their places are very dirty. They are a wealthy people, and their places look comparatively clean. * * * The Jains, owing to their aversion to taking life, are said to be infected with parasites."

A NEW SPECIES OF RAT FLEA.

As yet much evidence certainly has not been presented in favor of the theory that the most important intermediaries in the spreading of plague from rats to man are fleas from plague-infected rats. Even if rat fleas should have played an important rôle in the spread of plague in Sydney, as maintained by Tidswell and Thompson, their conclusions can not be generalized. How necessary it is to study this question in every place where plague occurs endemically or epidemically is shown by our observations of the rat fleas in Manila. Looking into this subject, we found, somewhat to our surprise, that the fleas infesting rats are not identical with those which have been described for other countries. Indeed, the species found here on rats appears to be new. Previous to the time when the writer began to collect rat fleas, Dr. W. B. Wherry, bacteriologist of this Laboratory, had already collected eight, which he kindly placed at my disposal. Thirty-four were subsequently caught. It is not easy to procure a large number of rat fleas, because, when the rodents have been caught, by the time they are killed and examined, the fleas have left them. For this reason Dr. Wherry succeeded in obtaining only eight fleas from fiftythree rats; while the thirty-four others were subsequently obtained from about one hundred animals. A few of the parasites were procured alive, were kept in a glass vessel for some time, and were then given an opportunity to bite both Caucasians and native Filipinos. In not a single instance did any of these fleas bite human beings. We have also collected a number of fleas from

¹Average mortality per 1,000 males in the plague epidemic of 1896 at Bombay, 2.63; per 1,000 females, 1.88; per 1,000 male Jains 8.69; per 1,000 female Jains, 6.77.

persons working in the old laboratory, where numerous animals were kept and where large numbers of rats were sent for examination for plague infection; these fleas proved to be *Pulex irritans* and *Pulex serraticeps*. The flea occurring on rats in Manila was never found on human beings.

I am indebted to Mr. W. Schultze, assistant entomologist of this Laboratory, for the greater part of the following description of the flea found on rats in Manila:

Pulex philippinensis, sp. nov., Manila, July 19, 1904.

Head, with very small bristles; front of head high and rounded. Eyes round. Above the latter and directed backward are the antennæ, which are club shaped and consist of three segments. First and third segments equal in size; the third segment cup shaped; the second segment very small. Superior maxilla elongated, triangular. Palpi maxillarum consist of four segments, of which the last is the longest. The posterior margins of the thoracic segments possess each a row of fine bristles, as do also the abdominal ones. On the latter the bristles extend from the back downward to the middle of the abdomen, and from below upward. On the back, on the posterior margin of the eighth abdominal segment are two large bristles. In the male, below the external genitals, two large bristles on each side—i. e., four in all. Abdomen of the male bent upward. In the interior of its abdomen are the spinally curved internal sexual organs, more or less distinct in different individuals. The abdomen of the female is oval and egg shaped. Bristle on the ninth segment around the external genitals. In the abdomen of the female between the seventh and the eighth segment is an intestine or sausage-like curved organ—the ovary. Behind it in one individual are several barrel-shaped ova.

The longest bristles are found at the lower ends of the femora. They measure 0.2 millimeter in the female and 0.15 millimeter in the male:

Color: Light reddish-brown.

Size of the female: Length, 1.8 to 2.67 millimeters; breadth, 0.80 to 1.25 millimeters.

Size of the male: Length, 1.16 to 1.78 millimeters; breadth, 0.70 to 0.75 millimeter.

Size of the ova: Length, 70 μ ; breadth, 55 μ .

Types in the collection of the entomological division of the

Biological Laboratory, Bureau of Government Laboratories, Manila, P. I.

Pulex philippinensis appears to be a stationary parasite of the rat; it is found in Manila. It is much like P. anomalus recently described by Baker and found by him in California. However, the head, eyes, antennæ, palpi, and bristles offer marked points of differentiation.

It is of interest to mention that Baker in his monograph on American Siphonaptera describes a new species of rat flea from Brazil, South America. It is a very large one, the male being 3.5 millimeters and the female 5.5 millimeters in length. Evidently there are several different species of rat fleas in different parts of the world.

EXPERIMENTS WITH FLIES.

Experiments which have been made to demonstrate that plague may be spread by flies are all inconclusive, because the arrangements were such that the transmission of the disease was inevitable. Flies were fed with bouillion containing plague bacilli and after varying intervals of time had elapsed were ground up. The emulsion so obtained was subsequently injected into guinea pigs or rats, which of course died of the disease. Such experiments can not determine with certainty whether plague can or can not be carried by flies.

Experiments were therefore conduct which were designed more closely to imitate what might take place in nature. The organs of plague cases, both from man and from guinea pigs, were kept in an anatomical jar, over which was placed a flytrap containing a number of flies. The door of the trap was then opened and the files allowed to come in contact with the plague organs in the lower vessel. After they had remained in the lower vessel for varying periods of time (from one to several hours) they were again induced to enter the trap by surrounding the lower vessel with some dark material, which caused them to seek the upper light space. The trap was then closed and placed over an especially constructed fly-proof cage, in which were two guinea pigs. The door of the trap was opened so that the flies could enter the cage. The guinea pigs had been shaved over an extensive area of the back and some sirup had been dropped on a few spots, to attract the flies and to cause them to come in contact with the bodies of the

animals. This experiment was performed twice; but in neither case did the guinea pigs contract plague. It had been proved by preliminary trials that the flies which had been allowed to come in contact with and to eat from the organs of plague cases had bacilli either in or on their bodies. Flies which had been allowed to come in contact with plague organs were then caught in the trap, chloroformed, picked up with sterile forceps and dropped into slightly alkaline bouillon; in every case a growth of plague bacilli was observed in the media in which they were placed.

Another observation, which was not made experimentally but under perfectly natural conditions, may here be recorded. The San Lazaro morgue at Manila, a building so constructed as to be insect proof, owing to the inclemency of the weather soon became in such a condition that flies could readily go in and out in large numbers. During post-mortem examinations on plague cases flies generally passed freely from the dissected cadavers to those making the examinations as well as to others present, and undoubtedly also to the outside of the building; yet no cases of plague have occurred in the part of the city where the morgue is located. However, this part of the town is only sparingly inhabited. Hence, no case can be attributed to flies as carriers of the infection.

When one takes what has been said above into consideration, one is certainly inclined to conclude that insects, as a rule, do not probably play a very important role in the spread of plague. However, the observation that this disease is exceedingly prevalent among that caste in Bombay which is probably more infected with pediculi than any other suggests that certain parasites may be operative in its transmission. As stated in the remarks introductory to this part of the paper, one of the cases here observed strongly suggests that pediculi may be carriers of the infection.

A CASE OF BUBONIC PLAGUE IN A CHILD, IN WHICH THE INFECTION WAS POSSIBLY CARRIED BY PEDICULI.

CASE No. 10. CERVICAL BUBO.

[Necropsy Protocol No. 910. C. S., a Filipina girl, 9 years of age, from Anda Street, Intramuros, Manila. Post-mortem examination five hours after death, on Saturday, March 5, 1904, at 4 p. m.]

The body of a female child, 9 to 10 years of age, well developed. Post-mortem rigidity strong. Post-mortem lividity well marked and extending over the sides of the body, being particularly notice-

able around the neck. The cervical glands on both sides are swollen, imparting a doughy sensation to the touch. The tissues in the neighborhood of the swollen glands are quite ædematous. The integument of the body shows no lesions. There is no discharge from the ears; nor does the external meatus on either side show any ulceration. The mucous membrane of the buccal and nasal cavities is congested, but otherwise normal. The scalp is infested with numerous pediculi, which seem to be ill at ease and run about as if disturbed. On section the superficial veins of the body discharge a moderate amount of very dark, fluid blood. The heart is normal, except that the superficial, subepicardial veins are much injected. The myocardium is somewhat soft, dull, and rather pale pinkish-yellow in color. The lungs are pinkish-purple. The lower lobes on the cut surface are dark reddish-brown, containing much dark, fluid blood and little air. Some of the areas seen on section are in a condition of almost red hepatization, containing a very large amount of dark, fluid blood. The mucous membranes of the bronchi are slightly swollen, hyperemic, and moist. The tracheal mucosa is in the same condition. The larvnx is much injected, particularly the epiglottis. The bronchial glands are dark purplish, slightly swollen, and somewhat softened. The spleen is of medium size. The capsule is smooth as a whole, though slightly wrinkled and grayish-blue in color. The cut surface is reddish-brown. The trabeculæ are well marked; the Malpighian bodies are fairly well visible. The mesenteric glands are reddish-purple, swollen, and rather soft. Both kidneys show fairly numerous subcapsular hemorrhagic areas, varying in diameter from about one-haf centimeter to a mere point. The capsules peel off easily, and after their removal the surfaces show alternating areas, either deeply injected or gravish-yellow. On section the vessels appear highly congested and the glomeruli stand out as deep reddish-yellow points. The tubules are gravish-yellow; the pyramids are deeply injected. mucous membrane of the pelves is smooth and slightly swollen, but not hemorrhagic. The substance of the kidneys is soft and almost gelatinous. The suprarenals are large, swollen, congested, and very soft. The surface of the liver is bluish-pink, mottled with pale grayish-yellow areas. The capsule is smooth. The cut surface is grayish-yellowish-pink. The veins contain a good deal of dark, fluid blood. The gall bladder contains a turbid, yellowish bile. The mucous membrane is smooth. The serosa of the stomach and intestines is injected. The superficial veins are marked as reddish lines. The mucosa as a whole is moderately injected, with punctiform hemorrhages in the ventricular portion. The intestinal lymph follicles are somewhat swollen. The peritoneal covering of the uterus and tubes is much injected. All of the fine superficial vessels are visible in consequence of the marked congestion. The cervical glands on both sides, including those along the sternocleido-mastoid muscles and the deeper submental ones, are enlarged, highly congested, and softened. On section a good deal of dark, bloody fluid can be scraped from the surface.

Anatomic diagnosis.—Hemorrhagic, acute, parenchymatous nephritis; congestion and œdema of the lungs; moderate fatty degeneration of the liver; hemorrhagic inflammation, hypertrophy, and softening of the cervical glands on both sides; more or less general hypertrophy of most of the lymph glands. Bubonic plague.

Smears from the cervical glands show numerous plague bacilli, but those from the spleen only a moderate number.

Before the body had been opened, three pediculi were picked up from the scalp with sterile forceps and dropped first into an empty sterile test tube and later into three flasks containing 50 cubic centimeters of sterile, slightly alkaline bouillon. All of the three flasks developed cultures of plague bacilli. The cultures were then transferred to various media and the bacteria were fully identified as typical plague organisms. One culture from the spleen and two from the cervical glands likewise developed plague bacilli.

Since this child, dead of bubonic plague, had come from a district which had been considered plague free for some time, inquiries were made as to the possibility of the girl's having been infested with pediculi from some one living in an infected district. Dr. R. E. S. Newberne, district medical inspector, reported on the matter as follows:

So far as the records show, only two cases of bubonic plague, prior to the one under discussion, have occurred on Calle Anda, the first in 1900 at No. 11, and the second in 1901 at No. 137. These numbers being at a considerable distance from No 89, and in opposite directions, it may be assumed that the district is not infected. The orphan, C. S., was taken to 89 Anda from the Hospicio de San Juan December 24, 1903, and remained in good health until the last days of February, when she became ill,

complaining of earache and fever, which did not yield to local treatment. The patient was sent to San Juan de Dios Hospital about March 4, where she died twenty-four hours later, after an illness of nine days. So far as can be ascertained, this child did not handle rats or do anything else to which infection could be ascribed. She slept on a petate on the floor of one of the upstairs rooms, as is the native custom. With the exception of the time from the 7th to the 16th of February, when she attended the public school on the corner of Victoria and Magallanes, she did not associate with children outside her home. The family assert that she was free from vermin when she was sent to the hospital, though it was admitted that Filipino children are generally infected with *Pediculi capitis*. Five bedbugs found in a crack of the floor upon which she slept and thirteen rats, only one of which was found alive, were sent to the Laboratory on March 11 for examination.

Mr. Chas. B. Hare, assistant bacteriologist in this Laboratory, who examined the rats, did not find any evidence of plague in them. Smears were made from the five crushed bedbugs, which likewise did not show any plague bacilli. It was intended to test the bedbugs by cultural methods, but this was overlooked by mistake.

Microscopic examination.—Sections from the cervical glands show, even on a superficial examination, a number of most profound changes, namely: (1) Almost complete loss of the normal structure and differentiation of the gland into cortical follicles and medullary cords; (2) advanced coagulation necrosis; (3) extensive extravasation of blood; (4) deposit of granular and fibrillar fibrin; (5) the presence of enormous solid, irregularly distributed masses of bacteria. The deeper portions of the cortex still present some oval compartments outlined by fibrous connective tissue, evidently once the trabeculæ; however, the latter do not contain normal lymph follicles, but masses of necrotic tissue and free extravasated blood. Where the dense masses of bacilli are located, there are few tissue elements left. The cells which are still recognizable as such are the mononuclears; their nuclei generally show a marked pyknotic condition. At the margins of the bacillar masses and clumps are mononuclear cells more normal in character, among which are found quite a few polynuclear eosinophiles. Almost as numerous as the latter are the plasma mast cells. The bacilli are found also in the tissues, next to the large colonies; here the micro-organisms do not form solid, dense masses, but are freely distributed among the cells. Intimately mixed with the leucocytic cells and the bacilli are numerous red blood corpuscles. The peripheral tissue,

next to the capsule, shows a dense infiltration with completely degenerated erythrocytes and contains hematoidin and hemosiderin. This zone appears as a part of the former lymph sinus; however, this can no longer be distinctly recognized as such. Here, likewise, numerous bacilli are found. Fibrin is quite irregularly and extensively distributed throughout the gland. In its interior it is observed to be in the form of a granular deposit and also in the shape of finer or coarser threads. Around the dense masses of bacilli it occurs in the form of fibrillar network, sending fine threads into the masses of micro-organisms. The tissue next to the capsule i. e., the former lymph sinus—likewise contains a network of fibrin. The small vessels are more or less completely occluded by hyaline (fibrin) thrombi, which are seen both in the interior of the gland and in the capsule. Here and there the fibrin extends from the interior of the vessel, through its wall, into the perivascular tissue. In the blood vessels bacilli, if found, are present in scanty numbers. Kidneys: The renal tissue presents a most striking picture. Sections from both kidneys, treated by Weigert's fibrin method, appear as if the vessels had been injected with a violet-stained gelatin. There is not a normal glomerulus to be seen. All the sections show a more or less complete obliteration by hyaline thrombi. most of the Malpighian bodies the hyaline thrombosis of the capillaries is so perfect that both the main branches of the afferent vessel and the smaller capillaries given off from the larger loops are sharply outlined. Most of the thrombi appear perfectly solid; however, some are hollow in the center, as can be seen both in transverse and in longitudinal sections. The endothelial lining of the thrombosed vessels is well preserved. Where the thrombi are comparatively thin, one can see, both in the transverse and in the longitudinal sections, endothelia which are perfectly normal to all intents and purposes. Nowhere do the thrombosed vessels to any extent show a loss of endothelia. Therefore, the thrombosis can not be attributed to a denudation of the vessels of their endothelial lining. The capsules of Bowman are likewise normal, though a few of them show a very moderate amount of thickening; their lining epithelium exhibits no marked changes. In some places the hyaline thrombi are continued into the vasa afferentia, and even into the vessels of which these are branches. Quite commonly there are seen between the uriniferous tubules parts of such small vessels filled with hyaline thrombi. However, none are found

in the larger arteries or veins, in some of which finely granular fibrin and desquamated endothelial cells are present. The vessel walls themselves show no damage aside from a minor degree of denudation of the intima. There is in particular no extension of the fibrin through the vessel walls, nor is there any evidence of mesophlebitic or periphlebitic—or arteritic processes. epithelia lining the convoluted uriniferous tubules are somewhat swollen, with indistinct outlines and a vacuolated protoplasm; but their nuclei are yet quite normal. A granular material partly fills some of the convoluted tubules. The epithelial lining of the straight tubules does not show any marked changes. The capsule of the kidney is normal. Nowhere do any of the renal blood vessels show a large number of plague bacilli; a few are possibly seen inside some vascular lumina; but even this is not certain. A moderate number of bacilli are seen in the lymph clefts between the tubules and around the Malpighian bodies. A few slender, long bacilli, which retain Gram's stain, are occasionally found in the tubules; but they are probably of no significance and represent an agonal or post-mortem invasion. Sections of the liver show a very few small periphlebitic inflammatory foci composed of small, round mononuclears. The liver cells all show a coarse vacuolation, some of the vacuoles being larger than the nuclei. The capillaries are moderately filled. There is no free extravasated blood. A very few plague bacilli are found between the liver cells.

CASE No. 11. RIGHT INGUINAL BUBO.

No complete necropsy protocol was kept. Body of a male Chinese, 26 years old, who died after an illness of seven days. Sections of the kidneys show hyaline fibrin thrombosis of the glomerular capillaries, with an extension into the afferent and efferent vessels as well as into the intertubular capillaries and small veins. There is general vascular dilatation and engorgement and cloudy swelling of the epithelia of the uriniferous tubules. In the spleen, which contains numerous plague bacilli, there is found a homogeneous, eosin-staining material, which is apparently derived from red blood corpuscles which have become confluent. At the margins of the homogeneous material erythrocytes singly and in groups may be distinguished. Coarse fibrin threads, forming a network, are here and there seen in the homogeneous material. Hyaline fibrin thrombi are likewise encountered in the small splenic vessels.

GROUP II. PRIMARY BUBONIC PLAGUE WITH SECONDARY PLAGUE SEPTICO-PYEMIA.

Case No. 12. Right Inguinal Bubo with Secondary Plague Septico-Pyemia.

[Necropsy Protocol No. 1011. M. N., Filipino male, 40 years old, from 77 Sacristia Street, San Nicolas. Ill six days; died early July 29, 1904. Post-mortem examination about six hours after death.]

The body of a middle-aged man, about 40 to 45 years old, in a fair state of nutrition, and strongly built. Post-mortem rigidity strongly marked, as is also post-mortem lividity on dependent parts. There are no wounds or abrasions. The right inguinal glands are swollen. Here the tissues are hard, infiltrated, and cedematous. Individual glands are not distinguishable. None of the other lymph glands, with the exception of the left inguinal ones, are palpable. Much dark, fluid blood escapes from the vessels when the body is opened. All the serous membranes are highly injected and reddened and numerous hemorrhages, to be more fully described, are seen. The pericardium, aside from congestion, is normal. The heart shows a number of subepicardial hemorrhages, varying from one to several millimeters in diameter. ventricle is well contracted and the right one dilated. The myocardium is rather soft, pinkish-yellow, and dull in appearance. Otherwise the heart is normal. The beginning of the aorta presents several raised, hard, atheromatous patches. The lungs are heavy and externally are bluish-purple, with black patches and subpleural hemorrhages. The right apex is adherent and consolidated, and at its very point there is an emphysematous cystic bladder of the size of a hazelnut with a smaller one of the size of a pea. These vesicles contain air and collapse on being cut in to. The apex as a whole is consolidated, and this area contains fibrous and calcareous nodules. The lungs, on section, are generally dark brown and contain much blood and ædematous fluid. The bronchial, tracheal, and laryngeal mucosa is moist, swollen, and deeply injected. The epiglottis is uneven and dark red in color. The papilla circumvallata of the tongue are much swollen. The spleen is enlarged to from two to three times its normal size and is firm in consistency. The capsule is smooth and steel-gravish-blue. The cut surface is smooth and rather light brownish-red. The pulp is not softened and does not protrude. The trabeculæ are easily visible; the Malpighian bodies are less so. The kidneys are normal

in size. The capsule is smooth and in general grayish-blue in color, with numerous and large subcapsular hemorrhagic areas. On section the vessels are engorged, the tubules grayish-yellow, and the surface dull in appearance. The pelves are smooth but highly injected, with several hemorrhagic spots. The ureters likewise show hemorrhages. The bladder is contracted and its wall firm; its serosa is highly injected. The mucosa is swollen, congested, and studded with small hemorrhagic spots. This organ contains 25 to 35 cubic centimeters of a coagulated, gelatinous, bloody material. Liver: The capsule is smooth and pinkish-purple, with a good deal of yellow mottling. There are confluent, subcapsular hemorrhagic spots on both sides of the insertion of the suspensory ligament. The consistency is increased, the elasticity decreased. The cut surface is ocher-yellowish-brown in color and very dull, as well as slightly uneven. The vessels contain much dark blood. The wall of the gall bladder is deep green with black hemorrhagic spots. It is thickened, edematous, and almost gelatinous. The viscus contains a moderate amount of thickened, almost black, pitchy bile. Stomach: The serosa is injected and shows a number of hemorrhagic spots. The walls as a whole are thickened. The contents of this organ consist of a dark coffee-brown, grumous mash. mucosa is swollen and all the vessels are injected. The whole surface is studded with small hemorrhagic dots. On the posterior wall, not far from the pylorus, there is a perfectly smooth cicatrix with radiating lines; however, this is not at all well marked and can be seen only on close and careful inspection. There is a fairly hard nodule, not larger than a pea, in the circumference of the pylorus; it is not well differentiated from the neighboring tissue, and shades off gradually. There is no ulceration in the region of the pylorus and the mucosa above the described nodule shows no changes beyond congestion, etc. The esophageal and duodenal mucosa likewise shows great congestion and hemorrhagic spots. In the large intestine there are found a number of dark, submucous nodules of the size of a pea; they contain dark, partly coagulated blood. The intestinal follicles are swollen. The tissues in the right inguinal region on being cut discharge a considerable amount of blood-tinged serous fluid. The glands are swollen, completely hemorrhagic, and generally indistinguishable as to outline. The capsules are only exceptionally recognizable, and as a rule the whole tissue forms one undifferentiated, bloody mass. The bloody infiltration is extensively continued into the surrounding tissue. The whole inguinal region is adherent to the overlying skin. The hemorrhagic condition is continued into the inguinal canal. The whole chain of glands, the right iliac, the retroperitoneal, the abdominal, the aortic, etc., is in a state of hemorrhagic inflammation. The areolar tissue of the left side of the pelvis and the abdominal cavity is ædematous, gelatinous, and extensively infiltrated with blood. The sheaths of some of the pelvic and abdominal vessels show hemorrhagic infiltration, as does also the loose tissue around the right kidney and the gall bladder. Except where specifically mentioned, the glands are all swollen, softened, and at least highly congested, if not hemorrhagic. Smears from the right inguinal glands show numerous plague bacilli. The organisms are also present in moderate numbers in those from the spleen.

Anatomic diagnosis.—Congestion and ædema of the lungs; hemorrhagic, acute, parenchymatous nephritis; parenchymatous and fatty degeneration of the liver; hemorrhagic inflammation of the right inguinal and many other lymph glands; extensive subserous, submucous, and interstitial hemorrhages. Bubonic plague.

The cultures inoculated at the post-mortem examination developed a typical growth.

Microscopic examination.—Upon a general survey, the glands of the right inguinal region show an extensive hemorrhagic infiltration with much necrosis, particularly in those places where the extravasaton is the greatest. The normal gland structure in general has disappeared, but here and there follicles may still be recognized where the blood extravasation is not so extensive. All vessels are much dilated and engorged. The connective tissue at the hilus is greatly increased. The capsules of the glands are loosened by a hemorrhagic ædema; and their cells as well as those of the connective tissue reticulum of the glands themselves are swollen and even completely necrotic. The periglandular, loose, areolar tissue is likewise hemorrhagic and in part distinctly necrotic. If the vessel walls are examined with a high power, it is seen that they are rarified by an ædematous infiltration. The cells forming them either show no nucleus at all or a poorly stained or a pyknotic one. The same changes are noticeable in the capsular and periglandular vessels. A number of the parenchyma cells, outside the zone of the dense bacillar zooglea masses, consist of fairly normal, small

mononuclars. Others of this type show a swollen protoplasm with either a poorly stained nucleus or one not stained at all. Bodies are also present which may best be described as cell shadows, which contain a rather fine, dark brown, granular pigment, probably a hemoglobin derivative. Coarse and fine granular, yellowish-brown pigment is also seen between the cells. Typical plasma cells, mononuclears with a large hyaline protoplasm, and polynuclears are not numerous. Eosinphiles are not seen. A few small vessels contain hyaline fibrin thrombi; but there is no extravascular fibrin reticulum present. Plague bacilli are found in dense masses, in which only a few autochthonous tissue cells are left and those greatly changed. From the dense clumps the bacilli infiltrate the spaces between the cells in those parts of the sections where more cells are still present. Spleen: The capsule shows no marked changes; the trabeculæ, on the other hand, generally show hyaline swelling and loss of nuclei. A small trace of an original Malpighian body is seen here and there. Most of the corpuscles have disappeared in consequence of necrotic processes, or at least their boundaries have become quite indistinct, the follicle losing itself in a dense mass of changed nucleated cells and degenerating erythrocytes. The pulp spaces are indistinguishable on account of their great engorgement with blood. Everywhere there is present a homogeneous mass with vacuoles, which are more or less occupied with nucleated cells. The homogeneous matrix, which stains well with eosin (in some places it takes the stain particularly well), is very probably a product of degenerated, agglutinated, confluent red blood corpuscles. In fact, in some places the more or less homogeneous, eosin-staining material is undoubtedly composed of red blood cells. The cells found in the vacuoles of the hemogeneous matrix are mostly polynuclears. Next in order of frequency come small mononuclear cells. These show more marked changes than the former, namely, poorly stained or very pyknotic or fragmented nuclei. Eosinophiles are also found. Plasma cells and large hyaline mononuclears are not seen in the spleen. Bacilli in small numbers are scattered all over the sections, and in places are found in more numerous groups. A few small vessels contain hyaline thrombi. Kidneys: All vessels are much dilated and engorged. The interstitial connective tissue is ædematous and shows a decided increase in some places, although not so great as that seen around the capsules of Bowman. In general the glomerular capillaries are like the

renal vessels, much dilated and engorged. Hyaline fibrin thrombi are found in few of the glomeruli. The thrombosis of the glomerulus is as a rule not complete and only a part of the tuft is closed by fibrin. The thrombi are sometimes continued into the vasa afferentia and efferentia and beyond them. The capsular epithelium shows a minor degree of degenerative, but no proliferative, change. The tubular epithelium is in a state of cloudy swelling and fatty degeneration. These degenerative processes are more marked in the convoluted than in the straight tubules. Most of the uriniferous canaliculi contain an abundance of granular material, while others have casts. Sections from the kidneys show an extensive infection with plague bacilli, which is mostly localized in the glomerular capillaries. Both in the open and in the thrombosed capillaries numerous bacilli may be seen in loose groups or sometimes even in dense masses. In the thrombosed vessels the bacilli are sometimes between the thrombus and the vessel wall. The organisms occasionally extend beyond the glomerulus into the vasa recta. Here and there bacilli are found at quite a distance from a glomerulus and occasionally in the capsular space and in the uriniferous tubules. Liver: The interacinous tissue shows small, round-cell, inflammatory foci and increase of the fibrous connective tissue. The portal and hepatic veins and the interlobular capillaries are dilated and engorged, showing a somewhat increased number of leucocytes. The parenchyma cells are very coarsely vacuolated and contain much bile pigment. Here and there plague bacilli are found in the capillaries; they appear in larger, denser groups near the central vein of the lobule and near the interlobular inflammatory foci. Where the bacilli are present extensively, there are seen cells with one, two, or three nuclei with many bacilli included in their protoplasm. These phagocytic cells appear to be vascular or lymphatic endothelial cells. Lungs: All vessels, including the interalveolar capillaries, are much dilated and engorged. alveoli are partly or completely filled with a granular, or almost homogeneous, eosin-staining material; they also contain quite a few desquamated, pigment-filled epithelial cells. Here and there extravasated blood is found in the air spaces. Fibrin is not visible in the pulmonary sections, nor are plague bacilli found. Stomach: The interglandular capillaries of the mucosa are greatly dilated, and free blood is observed between the glands. Towards the surface of the mucosa the hemorrhagic areas become larger. The most superficial parts of the mucosa, which are strongly infiltrated with blood, are necrotic. Here we find places where the surface epithelium is missing, so that the blood is free on the surface of the mucosa. The large parietal cells are very coarsely granular and stain deeply with eosin. Some of them have two or three nuclei, while others are much swollen and have lost their nuclei. Fairly dense masses of plague bacilli are found in some of the superficial hemorrhagic areas. The nodule in the region of the pylorus of the stomach shows a mucosa in an extensive state of glandular hypertrophy. The gland formation, however, is so typical that the process cannot be looked upon as an early stage of carcinoma. It is simply a glandular hypertrophy. In the duodenum the same vascular changes are seen as in the gastric mucosa. However, the blood extravasation is very insignificant and the vascular dilatation more moderate. In spite of this, the more superficial parts of the duodenal mucosa is necrotic, not uniformly so but in small patches. Eosinophilic polynuclear are numerous in the duodenal mucosa and submucosa. Plague bacilli are not found. dark cysts encountered in the interior of the large intestine are blood cysts formed between the muscularis and the submucosa of the bowel. The hemorrhage has completly dissected apart the muscularis and the submucosa. The contents of the cysts are pale, changed, or fairly normal erythrocytes, between which are found hyaline degenerated vessel walls and some irregularly distributed elastic fibers. The mucosa overlying the cysts is in a condition of coagulation necrosis. Plague bacilli are not found in or near these blood cysts; neither are any animal parasites encountered.

CASE NO. 13. LEFT SUBMENTAL BUBO.

[Necropsy Protocol No. 1027. F. C., a Filipina, 14 years old, from No. 195 Plaza Leon XIII, Tondo. Died after a short illness of unknown duration, on September 7, 1904, at 7.10 o'clock p. m. Post-mortem examination made on September 8, at 3 o'clock p. m.]

The body of a slender young girl about 15 years of age. Mammary glands fairly well developed and the pubes scantily covered with hair. The nutrition is good. There are no deformities, external wounds, sores, pustules, etc. The post-mortem rigidity is fairly strong; the post-mortem lividity is well marked on dependent parts and spreads toward the sides of the trunk. The left side of the face is quite cyanotic and swollen. The swelling is most marked underneath the parotid region; it is not sharply

defined but shades off gradually into the surrounding tissues. The swollen area is ædematous and doughy to the touch. The enlarged glands can not be distinguished well by palpation. The abdominal cavity contains a small amount of fluid. The lungs are nowhere adherent. The pericardium is normal, as is also the pericardial fluid. The right ventricle of the heart is well contracted, the left one dilated. A few small subepicardial hemorrhages are found on the surface. The myocardium is fairly firm, pink with a very faint tinge of vellow and somewhat dull in appearance. Otherwise the heart and the large vessels are normal. The coronary vessels are much congested, the two sides containing chicken-fat clots and some dark, fluid blood. The lungs are inflated, heavy, and bluish-purple on the outer surfaces. The right lung shows a number of small subpleuritic hemorrhagic spots, most of which are found at the two adjoining surfaces of the middle and the lower lobes. On section the pulmonary tissue is found to be much congested and cedematous and dark brownish-red. The bronchi contain some foamy, viscid mucus and their mucosa is slightly swollen and injected. The injection of the bronchial mucosa is very marked and the small arteries and veins are quite visible. mucosa of the larvnx is in a similar condition. The internal surface of the epiglottis exhibits about half a dozen pin-head-sized submucous hemorrhages. The bronchial glands are quite markedly enlarged, softened, and congested, those on the right side being more so than the ones on the left. Posteriorly at about the middle of the trachæ there are found two lymph glands each of the size of a bean, which are swollen, soft, and much congested. The left tonsil is swollen, hyperemic, and slightly ulcerated. The spleen is about one and one-half to two times the size of the normal adult spleen. Externally it is bluish-gray and has a smooth capsule. In consistency it is quite soft and flabby. On section the surface is granular and the pulp is found to be very soft. The trabeculæ are distinct; the follicles are very indistinct. Much brown juice can be scraped from the surface. The kidneys are quite large and fairly firm. The capsules are smooth, and the external color is pinkish-gray. Both kidneys show a few subcapsular hemorrhagic spots, the largest one being of the size of a split pea. The capsules peel off easily. On section the surface is quite dull and grayishvellow, with injected blood vessels, which have a prominent yellowish-gray background. The glomeruli are visible as red

points. The pyramids are rather pale. The relation of the cortex to the medulla is normal. The pelves are injected but without hemorrhagic spots. The right ureter is somewhat dilated and about one and one-half times the diameter of the left one, which is normal in size. The bladder contains about two ounces of turbid, vellow urine. The mucosa of the bladder and the ureters is hyperemic but not hemorrhagic. The ovaries, the tubes, and the uterus are normal. There are no fresh corpora lutea, but the uterine mucosa is diffusely hemorrhagic. The serosa of the corpus and the tubes shows dilated and congested vessels. The suprarenals are dark brownish-yellow, fairly soft, somewhat swollen, congested, and ædematous. The gastric and duodenal mucosa is enormously congested, the former showing extensive hemorrhages in the form of densely crowded dots and irregular areas which have evidently been formed by petechiæ and ecchymoses which have become confluent. The submucous hemorrhages in the duodenum are moderate in extent. The mucosa of the small as well as of the large intestine shows a marked hyperemia, but the lymph follicles are not swollen or at most very moderately. The mesenteric and retroperitoneal glands show only very moderate swelling and congestion. liver is of normal size. The capsule is smooth and purplish-blue with grayish-yellow mottling. The consistency is perhaps somewhat increased. On section the surface is even and vellowish-pinkishbrown. The distended veins of the liver contain much blood. In the center of the right lobe there was found a focus the size of a pea, composed of a somewhat loose, soft, grayish-white mass. The gall bladder is normal and contains some dark greenish-yellow bile. The ducts are normal. The pancreas is slightly swollen but otherwise normal. When the swollen region of the left side of the face is incised, a yellowish serous fluid escapes from the areolar subcutaneous connective tissue. The skin is firmly adherent to this tissue and can be dissected only with some difficulty. The color of the serous fluid is yellowish with a faint tinge of red. On dissecting deeper into the tissue, enlarged lymph glands are encountered, which are swollen, soft, and much congested. Glands are met with deeper down which are intensely so. The hemorrhages, however, do not extend into the ædematous periglandular tissue. The parotid gland is swollen and much congested, but not hemorrhagic. Brain: The longitudinal sinus contains a large chickenfat clot. The pial vessels both on the convexity and at the base

are enormously congested. The brain substance shows many hemorrhagic points (small hyperemic vessels); otherwise no noteworthy changes are seen.

Anatomic diagnosis.—Congestion and parenchymatous degeneration of the kidneys; congestion and ædema of the lungs; one necrotic focus of the liver with congestion and fatty degeneration; multiple subserous and submucous hemorrhages; left submental hemorrhagic bubo. Bubonic plague.

Smears from the deep hemorrhagic submental glands show many typical plague bacilli; while those from the superficial, softened, and congested glands reveal only a very moderate number. In the juice of the spleen there are very few, and in that of the lungs they are exceedingly scanty. Culture tubes inoculated from the hemorrhagic glands and from the spleen developed a typical growth.

Microscopic examination.—Sections from the deep submental glands show a complete loss of the finer structure, extensive free hemorrhages, and the formation of a homogeneous eosin-staining material (evidently derived from degenerated blood corpuscles . which have become confluent, and extensive areas of necrotic material. In the latter there are a few cells with pyknotic nuclei. Vessels are still recognizable in this necrotic mass, but merely by faint outlines, the greatly dilated walls having become much thinned and loosened. The capsule has been loosened by a cellular infiltration composed of mononuclear and polynuclear leucocytes. The infiltration extends beyond the capsule into the loose, periglandular, areolar tissue. In the outer part of the gland, the cells are better preserved and the hemorrhagic extravasation is moderate. The cells found here, aside from erythrocytes, are mononuclears and a few polynuclears. The former are mostly of the small type, although there are some large ones, with a vesicular nucleus containing a reticular chromatin and a large body which has a marked affinity for methylene blue. Bacilli are found irregularly diffused throughout the gland; they are very poorly stained, shelllike, and oval. The more superficial cervical glands are fairly well preserved in structure, showing an increase of the hilum connective tissue, great dilatation of the otherwise intact vessels, very small, scanty areas of blood extravasation, and large mononuclears with a somewhat basophilic protoplasm. Bacilli are present in very scanty numbers. No fibrin thrombi or fibrin networks are found in any of the glands. The spleen shows small, not well defined

corpuscles and indistinct pulp spaces crowled with red blood corpuscles and nucleated cells. Most of the latter are small mononuclears. Large mononuclear, lymphatic endothelia are not very numerous, a few of them containing the remnants of red blood corpuscles. A moderate number of plague bacilli are present in the sections. The vessels of the kidneys, including the glomerular capillaries, are greatly engorged and small areas of blood extravasation, both subcapsular and interstitial, are seen here and there. The uriniferous tubules show a loss of epithelium and advanced cloudy swelling with granular material in the tubular lumina. The capsules of Bowman are not thickened. There is no proliferation of the glomerular epithelium. The interstitial connective tissue is ædematous and here and there shows some finely granular material deposited between the tubules. Plague bacilli are not seen in the renal sections. The suprarenals show an enormous engorgement of the interfascicular capillaries. In the liver one sees great dilatation of the capillaries, and veins with an increased number of leucocytes in the former. The parenchyma cells are both finely and coarsely vacuolated. One of the three pieces of of liver which was taken for microscopic examination shows a very small focus of coagulation necrosis. Here the parenchyma cells are completely necrotic and in fact indistinguishable. A few small mononuclears are found scattered over the necrotic focus, which also shows a reticular, finely fibrillar matrix. At the very margin of the focus is seen a multinuclear giant cell, with a crescentic arrangement of its nuclei. The large cell has the typical appearance of a giant cell in tuberculosis. There are also encountered in this area a very few cells approaching the type of the epithelioid cells of the bacillar tubercle. The larger necrotic focus found in the center of the right lobe likewise consists of cells in a state of complete coagulation necrosis and finely granular material. At the periphery of the mass there is a zone of cellular infiltration composed of small lymphoid cells, mixed with considerable numbers of ordinary polynuclears. Eosinophiles are not seen. In the interior of the necrotic mass there are found a number of reticula of typical fibrin which gives Weigert's reaction. It is in connection with this fibrin and in its neighborhood that great numbers of shell-like plague bacilli are encountered. No plague bacilli are found in other parts of the hepatic sections. Neither in the small tubercle nor in the larger necrotic focus could any

tubercle bacilli be demonstrated. In the lungs the vessels are dilated and the alveoli are mostly open, some of them containing desquamated epithelia, others fairly numerous red blood corpuscles, and still others a hyaline or finely granular material, evidently the product of an ædematous fluid, coagulated by the fixing and hardening liquid. No plague bacilli are found in the pulmonary sections. The gastric vessels are dilated and greatly engorged. Much blood extravasation has taken place from the interglandular capillaries. The petechiæ and ecchymoses are in the outermost layer of the mucosa and often separated from the surface only by a thin remnant of tissue. The other zone of the mucosa towards the free surface is necrotic and its cells are swollen and have lost their nuclei. Numerous plasma mast cells are seen in the submucosa. Plague bacilli do not appear in the areas of blood extravasation, nor anywhere else in the stomach wall.

Case No. 14. Right Cervical Bubo with Secondary Plague Septico-Pyemia.

[Necropsy Protocol No. 889. E. J., Filipino, male, 63 years old, from No. 142 Caballeros Street, San Nicolas District. Died February 18, 1904, at 1 o'clock p. m. Sick five days; cause of death unknown. Post-mortem examination made February 19 at 8.45 o'clock a. m., about twenty hours after death.]

The body of a native well advanced in years; hair gray. Postmortem rigidity still fairly well marked, but beginning to disappear. The post-mortem lividity is pronounced on dependent parts and spreads toward the sides and interior surfaces of the body. The integument shows no injuries or sores. There are no buboes externally visible in the inguinal, cervical, or any other region. The abdomen is slightly distended. On section some dark, fluid blood escapes from the severed veins. The peritoneal and pleural cavities contain a small amount of serous fluid, the serous membranes are somewhat dull and their vessels markedly injected. The pericardium contains a small amount of clear fluid. The heart muscle is soft and flabby; the left ventricle is moderately contracted, and the right one dilated. The external surface shows a number of small irregularly distributed subepicardial petechiæ. The myocardium is pinkish-yellow. The valves are normal. The arch of the aorta is atheromatous. Lungs: Both apices are adherent, the adhesions being firm and fibrous. Externally the lungs are dark purplish-blue in color; on section they are dark purplishbrown. They are rich in dark, fluid blood, but do not contain much

air. The bronchi show a swollen and congested mucosa, and contain a moderate amount of viscid mucus. The mucosa of the trachea and larynx is congested, particularly that of the epiglottis. The spleen is small and its capsule slightly wrinkled and rather soft in consistency. On section the trabeculæ are well marked, the Malpighian bodies are indistinguishable and the soft pulp soft and dark-brownish-red. The kidneys are normal in size; their capsules are smooth, rather dull, and purplish-blue. On section the surface is gravish-white, and the vessels considerably injected and prominent. The mucosa of the pelves is smooth and hyperemic. Liver: The capsule is smooth and purplish-blue with some grayishwhite. On section the veins discharge a good deal of blood; the surface is smooth and dull brownish-vellow. Here and there a pale gray, soft necrotic focus of small size is seen. The gall bladder and ducts are normal. The gastric and intestinal mucosa is hyperemic, the former showing a moderate number of small hemorrhagic spots. The lymph glands in general are not much changed, except the right superficial cervical glands, which are swollen, soft, ædematous, and highly congested, but not diffusely hemorrhagic.

Anatomical diagnosis.—Congestion and cedema of the lungs; congestion and parenchymatous degeneration of the kidneys; subserous and submucous hemorrhages; right cervical bubo. Plague.

Smears from the cervical glands of the right side show numerous plague bacilli as well as numerous delicate, small streptococci. Culture tubes inoculated from the cervical glands and from the spleen developed a typical plague growth. No streptococci were found in the cultures.

Microscopic examinations.—The capsule of the cervical glands is thickened, and the connective tissue at the hilum much increased. The vessels of the capsule and the hilum are dilated and much congested. The entire substance of the glands is traversed by dilated, densely filled vessels; the differentiation into follicles is almost completely lost, the medullary cords are no longer distinguishable. The peripheral lymph sinus is dilated and contains a number of mononuclear cells and a good deal of granular material. The blood vessels, though dilated and much congested, show no marked changes in their walls, except some thickening of the adventitia; they do not contain any fibrin but are densely crowded with

erythrocytes, and show an increased number of leucocytes. The cells of the lymphatic tissue proper consist of small mononuclears of the ordinary type and a considerable number of plasma mast cells. Eosinophiles are not seen. Great numbers of plague bacilli are found in the lymph sinus, in the other distinct lymph channels, vessels, and clefts and all over the gland substance. The interior of the most of the blood vessels generally is free from bacilli, although some of the vascular lumina show these organisms in considerable numbers. Gram's stain shows no streptococci in this gland, though smears made from the cervical glands at the post-mortem examination contain typical streptococci. Spleen: The trabeculæ and the walls of the splenic arteries are thickened; some of the Malpighian corpuscles are still recognizable, although most of them can no longer be distinguished, even those which are preserved being quite small. In general, corpuscles and pulp form one almost structureless mass consisting of a large number of red blood corpuscles and mono- and polynuclear cells. original pulp spaces can no longer be outlined, and all of the splenic tissue is profusely infiltrated with plague bacilli; however, these are not found in large solid masses, as in the lymph glands, but in small groups. The kidneys show profound parenchymatous degeneration. The lining epithelia of the convoluted tubules have generally lost their nuclei; the cells as a whole are much swollen and indistinct in outline; and the tubular lumina are more or less obliterated by granular material. The straight tubules show the same changes, though to a much less degree. The glomeruli do not show such profound changes, and are fairly normal in appearance, though quite a number of plague bacilli are found in some of them. However, these organisms are located, as it appears, not in the glomerular capillaries but between the loops of the tufts. The lymph clefts between the tubules likewise contain plague bacilli, but in moderate numbers only. The liver shows some very small interlobular inflammatory foci composed of small round cells, and here and there some increase in fibrous connective tissue around the interlobular vessels and bile ducts. The parenchyma cells are finely vacuolated. The capillaries are rather distended but not well filled with blood. In general the hepatic capillaries show very few plague bacilli, except in some places-i. e., those corresponding to the necrotic foci seen at the autopsy, where we find enormous sausage-like masses of these organisms. They are located in the capillary lumen, none being seen inside the cells. The parenchyma cells in the neighborhood of these bacterial emboli show evidence of necrobiosis (poorly stained nuclei, etc.). The pulmonary tissue exhibits alveoli partly filled with desquamated, pigment-containing alveolar epithelia, and granular material, while here and there may be seen an air space almost completely filled with blood. The interalveolar capillaries and the other pulmonary blood vessels are much congested and densely filled with blood. Quite a few plague bacilli are found in the interalveolar connective tissue. It appears that these organisms are located inside the epithelial cells lining the lymph clefts, some also being found inside the alveolar epithelia. None are found in the pulmonary blood vessels. The alveoli show the presence of a small number of large, slender bacilli, which, like the organisms of plague, are decolorized by Gram's method.

CASE No. 15. RIGHT AXILLARY BUBO.

[Necropsy Protocol No. 973. F. A., Filipino, male, age 28 years, from 661 Calle Bilibid, Santa Cruz; died May 9, 1904, at 11 o'clock p. m. Post-mortem examination made fifteen hours after death.]

The body of a strong, well-developed man between 25 and 30 years of age. Nutrition is good. Post-mortem rigidity is marked; post-mortem lividity is well developed. Near the right nipple there are seen two dried-up vesicles, which are completely collapsed and covered with dry epidermal scales. Otherwise there are no open wounds on the body. The right axillary lymphatics form a flat doughy mass of the size of the palm of the hand. The swelling in the right axillary space is not well defined but gradually shades off into the surrounding tissue; the skin overlying this region is very cyanotic. On being cut the mass is found to be very ædematous and it first discharges a blood-tinged serum and then a bloody fluid. No individual glands can be mapped out, but everything is completely infiltrated by a hemorrhagic exudate. The latter is continued into the deep fascia of the thorax and the pectoral muscles, finally reaching the intercostal muscles and penetrating into the thorax. The inguinal, cervical, and cubital glands are all to a certain extent palpable, and, when dissected out, are found to be somewhat enlarged, softened, and congested. On opening the body cavities the serous membranes are seen to be congested and rather dull. The serous fluid in the abdominal and thoracic cavities is not increased. The right lung has formed a few slight adhesions. On the right side there is found an area of subpleural hemorrhage, which forms a direct continuation of the ones radiating from the axillary bubo. The lungs are in general normal in form, fairly well inflated, and purplish-blue in color, with a few small subpleural hemorrhages. The pulmonary tissues are congested and ædematous. The mucous membrane of the trachea, the bronchi, and the larynx is somewhat swollen and highly congested. The heart is normal in size. The coronary vessels and their branches are much engorged. A few subepicardial hemorrhages are found on the anterior surface. The myocardium is rather soft and flabby and pinkish-yellow in color. Otherwise the heart is normal. The spleen is twice its normal size, quite soft, steel-gray externally, and reddish-brown on section. The pulp is quite soft. The trabeculæ are well marked, the Malpighian bodies less so. The liver is normal in size, though somewhat increased in consistency. It is purplish-blue in color with some gravish-white mottlings. On section it is found to be much congested. The gall bladder is normal, containing a large amount of yellow, thin bile. The kidneys are normal in size, smooth, bluish-purple externally, and yellowish-pink on the cut surface. The pelves are much injected, that of the left kidney containing a small amount of extravasated blood. The adrenals are normal in size, of fair consistency, and brownish-vellow in color. The mucosa of the stomach shows numerous small hemorrhagic spots, which are also seen in moderate numbers in the duodenal mucosa. The lymphatics of both the large and the small intestine are somewhat swollen.

Anatomic diagnosis.—Congestion and cedema of the lungs; parenchymatous degeneration of the kidneys; right axillary hemorrhagic bubo; general swelling, hypertrophy, and congestion of the lymph glands. Bubonic plague.

Smears made from the different organs show plague bacilli, which are present in considerable numbers, together with some diplococci in those from the axillary hemorrhagic bubo, and in fair numbers in the left inguinal glands and in the liver, while in the spleen they are plentiful. The cultures developed typical plague bacilli, but all were contaminated.

Microscopic examination.—The right axillary glands show an almost complete necrosis with a loss of all the finer details of structure and an intense hemorrhagic infiltration, which extends into the surrounding tissue. A considerable number of very poorly

staining plague bacilli are seen in the sections. The inguinal glands exhibit an increase of connective tissue at the hilus, great cedema, general vascular dilatation, and engorgement with hyaline degeneration of the rarefied vessel walls. A moderate number of plague bacilli are present in the inguinal glands. In the spleen the Malpighian corpuscles are small and the pulp spaces are not very distinct, being crowded with many polynuclears. The erythrocytes are only moderately numerous. The lymphatic endothelia of the pulp spaces have proliferated to a moderate extent, some of them showing two or three nuclei. The splenic sections contain innumerable plague bacilli; however, they are not present in such solid zoogleal masses as are frequently seen in primary buboes, but in very dense groups consisting of many hundreds of bacilli, between which the tissue cells are only sparingly seen. This condition is most marked in the peripheral lymph sinus. In the pulp spaces the bacilli are less numerous, though present to a large extent. A great condensation of bacillar masses is also seen at the peripheries of some of the Malpighian corpuscles, while their interior is almost entirely free from microbic invasion. Phagocytic cells containing bacteria are not seen in the spleen. The kidneys show a most profound degree of cloudy swelling of the tubular epithelia, with the presence of much granular material and some hyaline casts. All the vessels are much dilated and engorged, particularly the glomerular capillaries. Here and there in the glomeruli an incomplete fibrin thrombosis is met with. Groups of plague bacilli are found in the glomerular and intertubular vessels, some of them amounting to fairly dense masses of bacteria. A very few isolated bacilli are seen here and there in the uriniferous tubules. The hepatic sections exhibit veins and capillaries much dilated and engorged with an increased number of leucocytes. The parenchyma cells are vacuolated and coarsely granular, their nuclei often being poorly stained and even lost. The capillaries show small, loose groups of plague bacilli, but nowhere a complete occlusion by dense bacillar masses, as seen in other cases of bubonic plague, with metastatic bacterial emboli of the liver. In the pulmonary sections the veins and the interalveolar capillaries are greatly dilated, and the latter here and there contain small, loose groups of plague bacilli. Very few single bacilli are seen in the alveoli. Here and there they are in contact with the wall, so that their derivation from the intracapillary groups is probable.

GROUP III. PRIMARY BUBONIC PLAGUE WITH SECONDARY PLAGUE PNEUMONIA.

CASE No. 16. AMBULATORY PLAGUE, OR PESTIS MINOR.

It has been known for many years, long before the specific bacillus had been discovered, that plague cases exist in which the symptoms are very mild and in which the patient may be free from any marked elevation of temperature. These have been called ambulatory plague or pestis minor and have quite properly been compared to typhus ambulatoria.

Griesinger, Liebermeister, Montague-Lubbock, Manson, Scheube, and others have called attention to the fact that such ambulatory cases, in spite of the mild character of their symptoms, are liable to sudden collapse and fatal termination. Manson (Tropical Diseases, 3d edition, London, 1903, p. 249) speaks of this type as abortive or larval plague, and states that certain epidemics are distinguished by a larger proportion of mild cases. "In such," he says, "buboes form and suppurate or resolve, the associated constitutional symptoms are comparatively mild or perhaps altogether wanting. In every epidemic there may be cases in which the patient is able to be about, having little, if any, fever, and apparently being little inconvenienced by the disease. Such cases, however, may collapse suddenly."

However, there is very little to be found in literature showing that a more careful investigation of this type of cases has been made. So scanty is our knowledge that the extensive report of the Indian Plague Commission has no more to say about the pathology of pestis minor than the following (Vol. V, p. 432): "Death from pestis minor probably never occurs, but, at any rate, no description of the pathology of plague deals with this type."

The case to be reported as an example of ambulatory plague demonstrates that the term "pestis minor" is a misnomer, when applied to such "walking-plague patients" as die suddenly. In our case the necropsy and the microscopic examinations furnished evidence sufficient to account for the unexpected death in the absence of any marked previous subjective symptoms of ill health. The history and the findings in this case, which is somewhat complicated by a simultaneous, evidently very recent, tuberculosis of the lungs, were as follows:

The death of a Filipino lad, 17 years old, was reported to the Santa Cruz board of health station, Manila, P. I., on February 27, 1904, at 11 o'clock a.m. The body of the deceased was found in a dimly lighted loft in the corner of a lower unpaved room, adjoining a soda-water factory at No. 185 Calle Misericordia. On

examination nothing worthy of note was seen, except enlargement of the glands in the inguinal region and Scarpa's triangle on both sides, as well as a chronic skin affection of both legs. The position of the body had been changed since death. The boy had been employed for general purposes about the place for from four to five months, during which time he appeared to be in good health, except that for some weeks before his death his face was rather pale, and he did not sleep very well. He spent the evening of February 26 playing in the street with other boys until 11 o'clock, when he went to bed. About 12 o'clock he awoke, complaining of pain in the chest and difficulty in breathing. His condition soon became alarming, and a native physician was sent for, who was unable to do anything for him. At 2 o'clock he was dead. (History furnished by district medical inspector, Dr. Terry, of the Board of Health.)

CASE No. 16. Ambulatory Plague, Terminating by Embolism of the Pulmonary Artery.

[Necropsy Protocol No. 901.]

Post-mortem examination made on February 27, 1904, at 3 o'clock p. m. The body of a Filipino boy, about 17 to 18 years of age and well developed. Post-mortem rigidity strongly marked; post-mortem lividity prominent on dependent parts and extending over the sides of the trunk and neck as well as over the anterior surface of the latter. A greenish-brown, foamy, ill-smelling fluid oozes from the anterior nares. The anterior surface of the lower extremities, from the ankles upward to about midway between the knees and Pupart's ligaments, is covered with a vesiculo-pustular eruption. The lowermost portions of this eruption consists of shallow ulcerations covered with brownish, bloody scabs. skin lesions higher upon the thigh are still purely vesicular and the collopsed vesicles are covered with epidermal scales. chain of lymph nodes below Pupart's ligaments on both sides is swollen, the most marked swelling being found in the lowermost glands on each side. The swollen region is soft and doughy. However, no fluctuations are noticeable. On incision of the skin the superficial veins discharge a rather small amount of dark, fluid blood. The pericardium is smooth and normal, and contains a small amount of clear, straw-colored fluid. The visceral layer of the pericardium shows dilated and congested veins. On the posterior surface, over the left auriculo-ventricular zone two or three dozen hemorrhagic areas are seen, varying in size from a pin-head to a millet seed. Otherwise the visceral pericardium is normal. Heart: The myocardium is fairly firm and somewhat pale. The left ventricle is contracted, and the right one dilated. The latter contains a rather firm, though somewhat gelatinous, reddish-gray coagulum, which does not completely fill the ventricular cavity. The coagulum is continued into the pulmonary artery, which it completely fills. Here it is firmer in consistency and decidedly more gravish in color. These variations from the consistency and color of the clot in the heart became greater the farther the distance from the entrance of the pulmonary artery to the interior of the ventricle. The thrombus extends into the main branches of the pulmonary artery, from where it can be followed into the lower, secondary branch of the right side, and it is then lost in the highly congested lower lobe of the right lung. A distinctly hardened infarcted area can not be found. The blood in the left ventricle and auricle is fluid and of a dark purple color. The lungs are quite firmly adherent to the parietal pleura. The lower lobe of the right lung is particularly firmly adherent to the diaphragm. The partly obliterated pleural sacs contain a small amount of slightly turbid, yellowish fluid. The upper lobes are dark grayishpink and contain a considerable amount of air; the lower lobes, particularly the right one, are dark purplish-blue and contain very little air. On section the lower lobes are found to be quite cedematous and filled with a very dark, purplish, fluid blood, which oozes out freely from the larger and smaller vessels. The right lower lobe on the cut surface shows some small, gravish-white dots. The bronchi contain a small amount of foamy, gravish-white fluid. Their mucous membrances are injected and rather bright-red in color. The trachea and the larynx likewise show swollen, injected mucosa. The spleen is large and bluish-pink in color, its capsule smooth and shining. The cut surface is dark purplish-brown. The trabeculæ and the Malpighian corpuscles are distinct. The pulp is fairly firm in consistency. The kidneys are rather small and their surface smooth and of a dark grayish-pink color. capsules peel off easily, and the denuded surfaces then show the small vessels to be much injected. They stand out plainly on a gravish-vellow background. On section the vasa recta and the glomeruli appear quite strongly injected and intensely scarlet.

The tubules are quite markedly yellowish-gray, the surface on the whole being rather dull. The pyramids are purplish. The mucous membrane of the pelves is smooth and somewhat congested. Liver: The capsule is smooth, shining, transparent, and pinkish-gray in color, some areas being decidedly mottled. The cut surface shows the center of the lobules to be grayish-white; they are distinct in outline. The surface as a whole is dull. The veins contain much dark, fluid blood. The gall bladder contains some turbid, greenishyellow bile, its mucous membrane being smooth. There are no stones; the ducts are normal. The stomach and intestines appear fairly normal. The suprarenals and the pancreas are normal. The inguinal glands on both sides are enlarged, swollen, soft, and rather bluish-pink in color. On section they show injected vessels, which stand out prominently on a grayish-yellow background. The substance of the glands is soft. No abscess formation has occurred. An abundant, grayish-white fluid can be scraped from the cut surface. The lower glands of the chains on each side are the largest, showing a central softening quite markedly. There is, however, no abscess formation. These glands are equal in size on both sides and measure 4.8 by 3.7 by 1.7 centimeters. mesenteric, the cervical, and the other glands examined are all moderately enlarged and more or less congested. Marked congestion exists in the bronchial glands.

Anatomical diagnosis.—Congestion and cedema of the lungs; parenchymatous degeneration of the kidneys; embolism of the pulmonary artery; inguinal buboes. Plague.

Smears were made during the post-mortem examination from the liver, the spleen, and the glands. In the smears from the spleen and the glands only a very small number of bacilli are found. They are more numerous in those from the liver. Some of the bacilli found in the latter are quite typical in appearance, showing polar staining and rounded ends. Other bacilli are swollen, somewhat irregular, and approaching the type of the involution forms seen on artificial media. Still others are small and look decidedly like diplococci. Others form small chains, the individual members of which appear to be in a state of partial dissolution. The cultures inoculated from the organs developed typical plague bacilli.

Microscopic examination.—Liver: The liver shows extensive interlobular, inflammatory foci, formed by a cellular exudate.

The infiltrating cells are found around the interlobular arteries, veins, and small bile ducts. The small interlobular vessels show a marked thickening of the adventitia, and most of the lumina are found to be surrounded by a number of concentric rings, composed of delicate connective tissue fibers. Occasionally there is found a small vessel entirely occluded by an obliterating proliferation of the lining endothelium. The cellular exudate of the inflammatory foci consists of lymphoid cells and a high percentage of eosinophilic polynuclears. In some places the latter form one-fourth to one-fifth of the total number of infiltrating cells. Plasma cells are very sparingly represented. While this description conforms in general to most of the inflammatory foci in the liver, other areas present decidedly varied appearances. In these the foci, which are more or less nearly circular, clearly show a center consisting of epithelioid cells with a vesicular nucleus and a large protoplasmic body. Multinuclear giant cells are found in some of the nodules. The histology of these round or oval nodules is clearly that of the bacillar tubercle. The nodules also show a fine, concentrically arranged, fibrillar network of connective tissue fibers. Regressive changes are not seen. There is no caseation, nor does Weigert's stain show any fibrin. The histology of the small tubercules in the liver, and it may here he added that of the similar structures found in the lungs, is identical with what has recently again been described by Baumgarten as being the structure of the tubercle experimentally produced and about two or three weeks old, which has not yet undergone any regressive changes, but in which the appearance of the first giant cells indicates that the prolifieration of the tubercle bacillus, as well as that of the inflammatory cells, has come to a standstill. Baumgarten also describes the occlusion of small vessels by endothelial proliferation brought about by the presence of the tubercle bacillus. The nodules in the liver as well as those in the lungs contain an element which is foreign to the typical uncomplicated tubercle, namely, numerous eosinophilic polynuclears. It is impossible to prove the presence of tubercle bacilli, but the tubercles are found to be infected with the organisms of plague. The latter are seen here and there in both types of inflammatory foci, in those which simply show the structure of an ordinary inflammatory area, and in those possessing the features of a bacillar tubercle. The parenchyma cells of the liver exhibit a moderate degree of fatty degeneration. The capillaries are well filled with blood. Lungs: Section from the right lower lobe show a great engorgement of the interalveolar capillaries; in fact, all the blood vessels are highly congested. The alveoli present a varying picture; some of them are open, having contained air only, and others are filled with extravasated blood, which does not show any degenerative changes. Desquamated alveolar epithelia, containing hematoidin granules, are seen occasionally. The unchanged character of the blood, the small amount of pigmentcontaining alveolar epithelia, and the absence of areas of coagulation necrosis, show that the blood extravasation must be of comparatively recent in date. Quite a few of the air spaces are filled with homogeneous coagulated material, which stains deeply with eosin. Other alveoli contain a lighter staining material. Neither the homogeneous nor the granular material takes Weigert's fibrin stain, nor do the capillaries contain any hyaline (fibrin) thrombi. Here and there is found a small solid nodule, hardly greater in size than the larger alveoli themselves. These nodules are composed of epithelioid and lymphoid cells and a considerable number of eosinophilic polynuclears. Multinuclear giant cells are likewise found in or near the center of some of the nodules. In fact, the latter are absolutely identical in their make-up with the tubercles which may be seen in sections of the hepatic tissue. Both in the pulmonary and in the hepatic tissue neighboring nodules are found, the peripheries of which are in contact with each other. However, the individual nodules have not become confluent and their outlines are well preserved. It is impossible to demonstrate tubercle bacilli in these nodules, but they show a small number of the bacilli of plague, found scattered between the cells. In properly stained sections it is seen that some of the alveoli contain enormous numbers of plague bacilli, such air spaces appearing almost filled with them. Small groups of scattered plague organisms can be found throughout the sections. Inguinal lymph nodes: The inguinal lymph nodes show numerous large, dilated vessels, replete with blood corpuscles. Around the larger vessels, particularly of the hilus vessels, there is a powerful development of the connective tissue. The general fibrillar connective tissue reticulum is everywhere much increased. The peripheral follicles generally show a well differentiated proliferation center. The peripheral lymph sinus and its branches are dilated. Eosinophilic polynuclears are found throughout the tissues, though nowhere in such large numbers

as in the lungs and liver. Bacilli are seen but sparingly in the sections. Kidneys: Sections of the renal tissues show practically normal glomeruli, moderate parenchymatous changes in the tubular epithelium, and highly congested vessels.

The noteworthy features of this case are its ambulatory nature, its sudden termination by embolism of the pulmonary artery, and its complication with what undoubtedly appears to be a very recent tuberculosis. According to Scheube tuberculosis is a very grave complication of plague; and Pearse likewise considers it a very unfavorable factor in this disease. It is very probable that in the case reported the plague virus first gained entrance through the skin lesions of the legs into the inguinal glands, where only a moderate multiplication occurred. The bacilli were then transported in the shape of a metastatic embolism into the lungs, from where the embolish grew until it obliterated the pulmonary artery. During the time these pathologic processes were going on, the symptoms were so mild that the plague-infected patient never stopped his regular work nor felt called upon to consult a physician.

GROUP IV. PRIMARY UNCOMPLICATED PLAGUE PNEUMONIA.

CASE No. 17. PRIMARY UNCOMPLICATED PLAGUE PNEUMONIA.

[Necropsy Protocol No. 970. A. Q., Chinese, male, 30 years old, a shopkeeper from No. 67 Tetuan, Santa Cruz. Sick six days; died May 18, 1904, at 10.30 p. m. Post-mortem examination made thirteen hours after death.]

The body of a well-developed Chinese about 30 years of age. Post-mortem rigidity moderately marked; post-mortem lividity well marked, extending to the anterior surfaces of the body. The skin as a whole is markedly cyanotic. The right axillary glands are slightly palpable; the other superficial ones are not. There are no injuries, wounds, or abrasions of the integument. On section, a moderate amount of dark, fluid blood escapes from the veins. The muscles are fairly moist and of a dark red (smoked meat) color. The serous membranes are injected and rather dull. The pericardium is much injected and contains a slightly increased amount of a yellowish somewhat turbid fluid. The heart is normal in size, the left ventricle contracted, and the right one dilated. At the sulcus a number of subepicardial hemorrhages are seen, the largest of which is about 0.5 to 0.6 centimeter in diameter. Both sides contain gelatinous post-mortem clots. The valves, the

endocardium, etc., are normal. The myocardium is of good consistency and reddish-pink, with a slight shade of yellow. The aorta and the coronaries are normal. Lungs: A few adhesions are found at the upper lobe of the right lung. The left pleural cavity contains an increased amount of fluid. The pleural surfaces are deep purplish-blue, particularly on the right side, the left side showing some admixture with grayish-white. On section, the right lung is found to be much congested and highly ædematous. The bronchi contain a fair amount of slightly blood-stained, frothy mucus, the alveoli only a small amount of air. The left lung is quite heavy, the lower lobe being entirely consolidated, and the greater portion of the upper one is in the same state. On section the consolidated pulmonary tissue is light brownish-gray. The cut surface is quite granular, and the juice which can be scraped off is bloody-gravish-vellow and quite turbid and opaque. In the upper lobe the consolidated area gradually shades off into the surrounding tissue and is not sharply defined. On a closer inspection both of the consolidated areas, in the upper lobes as well as in the lower show foci, varying in size from a pea to a hazelnut, grayish-yellowish-red in appearance, and surrounded by much congested, dark red areas. The bronchial glands are swollen, and very soft and dark, their contents being changed into an almost black, cheesy mass. The mucosa of the bronchi, the trachea, and the larynx are swollen and congested. The spleen is normal in size and flabby, with a wrinkled surface somewhat bluish-gray in color. On section the pulp is soft and protrudes over the surface, being dark reddish-brown in color. The Malpighian bodies and the trabeculæ are not very distinct. The kidneys are normal in size and their surfaces purplish-pink in color. On section, the vessels are much engorged, the tubules gravish-white, and the surface dull. The mucosa of the pelves is much injected. The suprarenals are swollen, softened, and brownish- purple in color. The liver is large and of increased consistency, its surface being purplish-blue with a great deal of grayish-white in some places. The cut surface is decidedly pinkish-yellow and as a whole dull. The vessels are much engorged. Stomach: The serosa shows a very few small hemorrhagic spots; the mucosa contains very numerous petechiæ and ecchymotic areas. The duodenal mucosa likewise shows some hemorrhagic spots. The lymphatics of the small intestine are somewhat swollen. The mediastinal, the mesenteric, the retroperitoneal, and the other superficial glands are all moderately swollen and congested. On section their vessels are found markedly engorged and their substance somewhat softened. The most pronounced enlargement is found in the axillary glands of both sides. Smears from the consolidated areas of the left lung show innumerable typical plague bacilli; those from the glands and blood only a few.

Anatomical diagnosis.—Parenchymatous degeneration of the kidneys; fatty and parenchymatous degeneration of the liver; multiple subserous and submucous hemorrhages; lobular pneumonia of the right lung. Plague pneumonia.

Cultures inoculated from the consolidated areas of the right lung developed a typical growth.

Microscopic examination.—Lungs: The alveolar spaces in the consolidated area are all more or less completely occluded. The distended air sacs, filled with a completely granular detritus, alternate in an irregular manner with the alveoli which are filled with a cellular exudate. The interalveolar septa are generally markedly broadened, however, this is not due to a marked increase in the cellular elements but to an ædema and hydropic swelling of the fibers and cells forming the interalveolar septa. The capillaries are greatly engorged and the walls of the veins are cedematous, loose, and frequently in a state of hyaline degeneration. Fibrin in finer or coarses threads is quite extensively seen, both in the interior of the alveoli and in the interalveolar tissue. The cellular exudate in the air spaces consist mostly of polynuclears with some mononuclears and desquamated epithelial cells. Erythrocytes are present everywhere, but in moderate numbers. Plague bacilli are found throughout the consolidated area, but not in very dense zoogleeal masses, though in great numbers in some places. In the renal tissue the most profound changes are cloudy swelling and fatty degeneration of the epithelia lining the uriniferous tubules. The interstitial tissue is quite œdematous, and the interstitial capillaries are filled ad mamimum. The glomerular vessels are likewise engorged, but otherwise do not show any profound changes. No plague bacilli are found in the renal tissue. The liver shows small, interlobular, inflammatory foci, composed mostly of mononuclear and embryonal connective tissue cells and a few polynuclears. However, these foci are quite small. The parenchyma cells show fine as well as coarse vacuolation. The interlobular capillaries contain a good

deal of blood. The spleen shows no marked histologic changes, and only a very few bacilli are seen after a prolonged search. Several lymph glands show marked dilatation and engorgement of their blood vessels as well as some ædema; otherwise they are fairly normal. The stomach exhibits greatly dilated vessels and capillary hemorrhages between the glands and upon the surface of the mucosa. No plague bacilli are found in the gastric areas of blood extravasation. In fact, none of the organs except the lungs contain any large number of these organisms. Outside the pulmonary tissue they are practically not found at all. The necropsy of this case shows it to be a pure plague pneumonia, without a general septic or septico-pyemic dissemination of the bacilli.

CASE No. 18. PRIMARY UNCOMPLICATED PLAGUE PNEUMONIA.

[Necropsy Protocol No. 971. C. C., Chinese, male, 27 years old, from No. 67 Tetuan, Santa Cruz. Sick six days; died May 18, 1904. Post-mortem examination fourteen hours after death.]

The body of a slender Chinese from 25 to 30 years of age. mortem rigidity well marked; post-mortem lividity moderate. surface is slightly cyanotic. There are no external wounds or ulcerations. None of the superficial lymphatics are palpable. section the veins discharge a good deal of dark, fluid blood. muscles are fairly moist and reddish-brown. The serous membranes are injected and dull. The pericardium is much congested, but the pericardial fluid is normal. The heart is normal in size, the left ventricle well contracted and the right one dilated. The anterior surface of the left ventricle, the apex on all sides, and the sulcus show numerous small subepicardial hemorrhages. Both sides contain gelatinous coagula. The myocardium is rather soft, pale, and reddish-pink. Otherwise the heart is normal. The aorta. and coronaries are normal. Lungs: The entire anterior and a part of the posterior surface of the lower lobe of the left lung is completely adherent to the pleura costalis. After the removal of the left lung it is found that the pleura pulmonalis of the lower lobe is thickened, yellowish, and covered with a thin fibrinous deposit. Both pleural sacs contain a slightly increased amount of fluid, on the left side this is turbid and contains some fibrinous flocculi. Externally the left lung is purplish-blue and the right one scarletpink. On section it is found that the lower lobe of the left lung contains a central area of pneumonic consolidation. In front this

extends as far as the pleura, and posteriorly and below it reaches to within about one centimeter of the pleural surface; but above there are still several centimeters of pulmonary tissue not yet in a state of consolidation. In the consolidated area the tissue is dark brownish-gray with lighter grayish-yellow spots. The cut surface is granular. The juice which is scraped off is turbid, grayish-white, creamy, and purulent. The area of consolidation is sharply defined from the rest of the pulmonary tissue, which is very much congested, dark brownish-red in color, and very œdematous, containing very little air. The upper lobe of the left side is congested and ædematous and contains much dark, fluid blood. The right side on section is found to be moderately congested and to contain quite a fair amount of air. The bronchial mucosa and that of the trachea and larynx are congested. The bronchial glands are swollen, softened, and dark purplish. The spleen is small and flabby and its surfaces wrinkled and bluish-gray. On section the darkbrown pulp is found to be softened, the trabeculæ visible, and the Malpighian corpuscles swollen. Kidneys are greatly congested. On section the vessels stand out very prominently on a very markedly grayish-yellow background. The surface is very dull. Liver: The surface is bluish-purplish-pink with gray mottling. The consistency is moderately increased. The cut surface is yellowish-brown and dull. The vessels are much engorged and the lobules enlarged. The gall bladder and ducts are normal. The gastric and duodenal mucosa shows numerous petechiæ. The petechiæ are so numerous and crowded in the mucosa of the stomach at the fundus that they have become more or less confluent at their margins. None of the superficial or deeper lymph glands, excepting the bronchial ones, show any marked changes.

Smears from the consolidated area of the lung show innumerable typical plague bacilli; others only a very few.

Anatomic diagnosis.—Parenchymatous degeneration of the kidneys and liver; multiple subserous and submucous hemorrhages; pneumonia; acute adhesive fibrinous pleurisy. Plague.

Microscopic examination.—Sections from the consolidated area of the lung show alveoli filled with mononuclear and polynuclear leucocytes, erythrocytes, and alveolar epithelia. The polynuclear leucocytes predominate considerably. Some of the air spaces show a coarse network of fibrin, though such places are not numerous. However, fibrin is found very extensively in the interalveolar capil-

laries and in some of the smaller pulmonary veins; it is present both in the shape of a reticulum and in that of tubular or solid thrombi. Here and there an extension of the intravascular fibrin into the vessel wall and into the perivascular tissue is encountered. The interalveolar septa in this case, unlike those in the preceding one, are not at all widened but rather delicate, excepting the space occupied by the distended capillaries. Bacilli are found extensively throughout the sections; here and there in a dense clump located in an alveolus and in other places distributed in such a way that it may be said that the cells of the exudate are embedded in a loose reticular matrix consisting of plague bacilli. In the renal tissue the tubular epithelium shows cloudy swelling, granular and fatty degeneration, and a loss of nuclei. Many of the tubules are collapsed and the interlobular connective tissue is ædematous The capillaries, includging those of the glomeruli, are congested, and a few microscopic areas of blood extravasation are found near the capsule. Sections of the liver show cloudy swelling and fatty degeneration of the parenchyma cells and great congestion of the capillaries and veins. However, there are no interstitial changes. The lymph glands exhibit engorgement of the blood vessels and lymph channels, some slight ædema, possibly some increase in fibrous connective tissue, and the presence of quite a number of plasma mast cells. Otherwise no changes have occurred. The vessels of the gastric and duodenal mucosa are greatly engorged, and some blood extravasation has taken place into the mucosa upon its surface. No plague bacilli are found in these small hemorrhagic areas; in fact, practically none of these organisms are found outside the pulmonary tissue. (The tissue from the spleen of this case had been lost.)

GROUP V. PRIMARY PLAGUE PNEUMONIA WITH SECOND-ARY PLAGUE SEPTICO-PYEMIA.

Case No. 19. Primary Plague Pneumonia with Secondary Septico-Pyemia.

[Necropsy Protocol No. 962. F. S., Filipina, female, 30 years old, from No. 148 Calle Anda, Intramuros, Manila. Died May 7, 1904, at 5.30 p. m. Cause of death unknown. Plague suspected. Post-mortem examination made sixteen hours after death.]

The body of a fat, stout woman, probably 40 years old. Postmortem rigidity moderate; lividity well marked. The posterior surface and the sides of the trunk, as well as the extremities, show numerous small petechiæ and ecchymoses, varying in size from one to several millimeters. The surface as a whole is cyanotic. On section the superficial veins discharge a good deal of dark, fluid blood. The serous membranes are highly injected and somewhat dull. The pericardium contains the usual amount of fluid, which is turbid and rather dark yellowish. The heart is somewhat enlarged, soft, and flabby. The left ventricle is very moderately contracted and the right one dilated, both of them containing clotted blood. The auriculo-ventricular openings are somewhat increased in size. The myocardium is soft and flabby, of a pinkish-yellow color, and easily torn. Otherwise the heart is normal. The lungs are adherent; the adhesions are easily broken up and are most marked on both the lower lobes and at the right apex. Both pleural cavities contain an increased amount of fluid of a turbid, slightly blood-stained character. The surfaces of both lungs are highly congested. The lower lobes particularly present numerous subpleural, hemorrhagic areas of an almost black purplish-blue color. Alternating with these are places where the surface is more grayishpink. The hemorrhagic areas protrude somewhat over the general surface, so that the latter is somewhat uneven. On section the pulmonary tissue is found to be much congested with dark, fluid blood in some parts of the lower lobes this amounts almost to complete red hepatization. Inside these very much congested areas are seen spots of the size of a pea or smaller, which are grayishred or more decidedly grayish-white. The lower lobes contain very little air, the upper ones a larger amount. The bronchial glands are not much swollen, though greatly congested, softened, and purplish-black in color, being somewhat hemorrhagic on section. The mucosa of the bronchi, the trachea, and the larynx is swollen and highly congested. The spleen is enlarged to about twice its normal size; its surface is bluish-purple and its capsule smooth and not very transparent. The cut surface is reddish-brown, uneven and granular, and the pulp protrudes. The trabeculæ are distinct; the Malpighian bodies are not. The kidneys are soft and almost gelatinous, their external surfaces being smooth, much congested, and deep purplish-blue. The capsules are transparent and peel off easily. On the cut surface the vessels appear highly engorged, the glomeruli stand out as deeply injected points, the tubules are grayish-white, and the surface as a whole is quite dull. The pyramids are much injected. The mucosa of the pelves is congested.

The suprarenals are swollen, soft, and dark brownish-yellow in color. The liver is large, of a somewhat increased consistency, and elastic. Its capsule is shining and transparent. The general color is pinkish-purple, but the areas of this hue alternate with places of a grayish-yellowish-pinkish color. On section the organ is found to be much congested. The hepatic veins discharge much dark, fluid blood. The surface is of a deep yellowish-brown and is quite dull. The acini are enlarged and the boundaries are not very distinct. Here and there a grayish-white point is seen on the cut surface. The gall bladder is distended, its walls edematous, and its mucosa swollen. The bile is viscid and dark greenish-yellow. The ducts are open. The serosa stomach and the intestines is much injected and rather dull. The mucosa of the stomach and the duodenum shows numerous small, hemorrhagic areas. The pancreas is normal. The uterus is markedly enlarged and rather soft. Its mucosa is softened, swollen, and hemorrhagic. The cavity, however, contains no free blood. The serosa of the uterus as well as of the appendages is much congested. The uterine muscularis is of good, firm consistency. The left ovary is enlarged to the size of a large walnut, nearly perfectly spherical, microcystic, and of a deep black-bluish-purple color. The right one is small, firm, and less congested. No fresh corpus luteum is present. The inguinal and cervical glands are slightly enlarged and moderately congested but not softened. The internal lymphatic glands, such as the mesenteric, the retroperitoneal, the iliac, and the mediastinal, are all very moderately enlarged and markedly congested, but neither markedly softened nor hemorrhagic.

Smears prepared from the different organs show the following: Lungs: Very many typical plague bacilli, also a number of very small diplococci. Spleen: A moderate number of plague bacilli. Liver: Quite a few bacilli. Heart's blood: Quite a few plague bacilli and some small cocci. Kidneys: Numerous plague bacilli.

Anatomical diagnosis.—Lobular pneumonic foci; congestion and beginning diffuse red hepatization of both lower lobes; general congestion of the lungs; subpleural hemorrhages; congestion and parenchymatous degeneration of the liver and kidneys; submucous hemorrhages in the gastric and intestinal mucosa; hemorrhagic endometritis; microcystic degeneration and congestion of the left ovary. Plague pneumonia and septicæmia.

Microscopic examination.—Lungs: Sections from the most congested portions of the lower lobes show all the blood vessels, including the interaveolar capillaries, to be engorged ad maximum. Numbers of air spaces are filled with a granular detritus including desquamated cells, some white and a few red corpuscles. Mixed with these elements are a moderate number of typical plague bacilli, which in some of the alveoli are present to a considerable extent. The majority of them are found in close proximity to the inner lining of the alveolus and not free in the center of the air space. Alveoli completely filled with innumerable plague bacilli are also seen. In such masses of bacteria one occasionally encounters a vessel broken into by these organisms; this observation refers alike to the capillaries, the veins, and the arteries. On the other hand, a few interalveolar capillaries are found to be completely thrombosed with densely packed plague bacilli. Alternating with alveoli, more of less completely filled, are air spaces which are open and even emphysematous, the interalveolar space having occasionally been ruptured. No fibrin is found in any of the alveoli. Kidneys: The most striking histologic change in the kidneys are metastatic emboli in the glomerular capillaries, completely filling some of the loops of the tufts. However, such emboli are found in a limited number of glomeruli only; nor are any tufts seen where all the capillaries are obliterated. The embolic closure is generally confined to one lobe of a glomerulus. Sometimes the embolic bacterial mass extends into the afferent or efferent vessel (it is impossible to decide which of the two is affected). The neighborhood of such thrombosed vessels shows small microscopic areas of blood extravasation, in which few bacilli are found. All the renal vessels are much engorged, particularly the small vessels, and the interstitial tissue is quite ædematous. Small microscopic areas of blood extravasation are encountered all over the sections. A few bacilli are often seen in such areas as well as in the tubules, the lymph clefts, and even occasionally in the small arteries and veins. The tubular epithelium shows profound cloud swelling and fatty degeneration. The interlobular capillaries of the liver exhibit the same metastatic emboli, composed of dense masses of plague bacilli, such as have been described above for the renal tissue. However, these bacterial thrombi are not very numerous, and in general the liver capillaries are free from bacteria. On the other hand, a small number of bacilli were seen in one interlobular vein. The liver parenchyma

cells show marked fatty degeneration. In the spleen the Malpighian bodies and the pulp spaces are not well marked, and the latter are densely filled. The proportion of leucocytes is increased. pulp spaces here and there show numbers of bacilli in fairly dense groups; but they are not nearly as crowded in the spleen as in the kidneys and liver. The gastric mucosa shows degenerative changes in the glandular cells, in most of which the nucleus stains poorly or not at all. Plague bacilli are found here and there in the loose connective tissue of the mucosa. The interlobular capillaries are enormously dilated, and blood is extravasated into the interglandular tissue and upon the free surface of the mucosa. Few plague bacilli are found in the œdematous and hemorrhagic interglandular tissue and in the areas of blood extravasation upon the mucosa. However, in general, the blood vessels do not contain any plague bacilli. The uterine mucosa is quite cedematous and its vessels much congested. Otherwise the uterus does not show any marked changes.

A consideration of the morbid anatomy and of the histopathology of this case shows that we are dealing with a primary plague pneumonia, in which the virus was very promptly carried by the blood current from the lungs to the distant organs. It is probable that the plague bacilli first entered the lungs by inhalation, multiplied in the alveoli, and invaded some of the interalveolar capillaries, where they formed solid thrombotic masses. Particles of the latter taken up by the blood current reached the kidneys, liver, spleen, etc., where in several instances they grew into completely obliterating emboli. It is a most remarkable fact that, in spite of the many bacterial foci interpolated into the vascular system, the general blood current did not become flooded with bacteria. In general the lumina of the vessels, both large and small, are found free from bacteria. Another point of great practical importance may be learned from a histologic study of this case, namely, that not merely the sputum but vomited matter, feces, and urine may be highly infectious.

GROUP VI. PRIMARY PLAGUE SEPTICÆMIA.

CASE No 20. PRIMARY PLAGUE SEPTICÆMIA.

[Necropsy Protocol No. 983. L. T. T., Chinese, male, 28 years of age, from No. 211 Santo Cristo Street, San Nicolas. Sick for ten days; died June 4, 1904, at 1 a. m. Post-mortem examination made fifteen hours after death.]

The body of a rather small, slender, young male Chinese, about 25 to 30 years old. Nutrition only fair, rather emaciated. The

skin is somewhat cyanotic. There are no ulcerations, wounds, or recent scars. Rigor mortis is absent; the post-mortem lividity is well marked. None of the external lymphatics are palpable. On section of the body a considerable amount of very dark, fluid blood escapes. The serosa of the stomach and intestines is very much congested and the small vessels stand out very prominently. The serous membranes are rather dull. A few hemorrhagic spots are seen in the peritoneal covering of the stomach and of the small intestines. The serous covering of the bladder likewise shows a few petechiæ. The abdominal fluid is somewhat increased, slightly turbid, and rather dark vellowish. The pleural sacs contain a normal amount of amber-yellow turbid fluid. Firm adhesions are found in the region of the apex of the right lung. The pericardium shows many engorged vessels, but is otherwise normal; so is the pericardial fluid in amount, color, etc. Heart: The left ventricle is well contracted, while the right one is dilated. The external surface shows several dozen irregularly distributed subepicardial hemorrhages, varying in size from a small punctiform dot to an area of 0.5 to 0.6 centimeter in diameter. The myocardium is of fair consistency and light pink with a little tinge of yellow. No further dissection of the heart is made, since it is to be photographed. The large vessels are normal. The lungs are expanded and heavy and the external surface is more or less deep bluish-purple. This color is most intense over the lower lobes, on which also a few ecchymotic spots are seen. On section the pulmonary tissue is brown in color and full of blood. In the left lower lobe there is some admixture with grayish-yellow, but there are no areas of distinct consolidation. A good deal of blood and serous fluid can be pressed out of the pulmonary tissue. The mucous membrane of the bronchi, the trachea, and the larynx is swollen and highly injected. The base of the tongue is much swollen and ædematous. The bronchial glands are quite dark. but not materially, if at all, increased, in size, or markedly softened. The spleen is enlarged to about twice its normal size; the capsule is wrinkled and gravish-bluish-purple in color. On section the organ is dark reddish-brown. The pulp is very soft and protrudes over the surface, which is quite granular. The trabeculæ and Malpighian bodies are distinct. Kidneys: The capsules are smooth and transparent and gravish-bluish-purple in color; they peel off easily. The external surface shows a few small subcapsular hemorrhagic spots. On section the glomeruli and straight vessels are much engorged, the uriniferous tubules grayish-yellow and the surface dull. The mucosa of the pelves is injected, showing a very few small punctiform hemorrhages; the ureters likewise exhibit a few petechiæ. The mucosa of the bladder is thickened and contains very numerous, densely crowded small petechiæ. suprarenals are swollen, soft, and yellowish-brown in color. The liver is large, with rounded margins. Its capsule is smooth and transparent and externally is bluish-purple, alternating with yellowish-gray. On section the surface is dull and grayish-yellow and the veins contain much blood. The consistency is markedly increased; the acini enlarged. The gall bladder is distended with a good deal of dark green viscid bile. The ducts are open; there are no stones. The mucosa of the stomach and the small intestine shows numerous small, irregular hemorrhagic areas. Both the superficial and the deep lymph glands are very little, if at all, enlarged, though they are markedly congested, particularly the mesenteric and the inguinal ones.

Anatomical diagnosis.—Congestion and œdema of the lungs; fatty degeneration of the liver; acute parenchymatous nephritis; multiple subserous and submucous hemorrhages (lungs, heart, kidneys, ureters, bladder, stomach, intestines). Plague septicæmia.

Smears from the different organs, including the glands, spleen, liver, lungs, etc., show a moderate number of plague bacilli. Tubes inoculated from the blood, the lungs, and the glands developed typical plague bacilli, but the cultures were contaminated by a small diplococcus.

Microscopic examination.—The histologic changes in the kidneys are very profound. There is universal cloudy swelling and fatty degeneration of the tubular epithelium. The tubules are generally filled with a granular detritus, and in many instances sharply outlined hyaline casts are found. These hyaline masses are composed of a homogeneous material (staining with eosin and somewhat with methylene blue); however, this does not give the tinctorial fibrin reaction. While the degenerative changes of the renal epithelium are most pronounced in the convoluted tubules, they are also present in the straight ones. The glomerular capillaries are generally not much altered, but here and there a partial hyaline (fibrin) thrombosis can be seen. Occasionally one observes an intertubular thrombosed vessel. Plague bacilli are found in small

groups in some of the glomeruli; they are also observed both in the capillaries and between them. A very few isolated organisms are encountered in the interstitial connective tissue between the tubules. The liver parenchyma cells show fine and coarse vacuolation. Interlobular inflammatory foci composed of small mononuclear cells are seen here and there, but the interlobular fibrous connective tissue is not increased. The interlobular capillaries and the vessels in general are much engorged with numerous, densely crowded red blood corpuscles. In a number of places inside the capillaries groups of plague bacilli are seen. However, this infection of the hepatic blood system is not general, the bacilli being confined to a number of more or less isolated spots. A good deal of granular bile pigment is found scattered throughout the hepatic tissue. The pulmonary sections show greatly engorged capillaries and veins, with an increased number of leucocytes in some of the latter. In a number of places the capillaries contain groups of plague bacilli. The alveoli contain desquamated epithelia and here and there a little extravasated blood. Otherwise there are no grave changes. No plague bacilli are seen in the alveoli. Spleen: The follicles are hazy in outline, the boundaries of the pulp spaces are indistinct and the latter are densely crowded with red blood corpuscles. The cells forming the follicles contain many large mononuclears with a large hyaline protoplasmic body. A considerable number of nuclei in the large hyaline mononuclears show a general pyknosis or lumping of the chromatin into dense granular masses. Plague bacilli generally are disseminated in moderate numbers throughout the splenic tissue, and in some parts of the pulp in larger groups. A very few of the smallest vessels contain hyaline fibrin thrombi. The inguinal lymph glands show much dilated and engorged vessels and an increase in the connective tissue at the hilum. Plague bacilli are seen here and there in little isolated groups. In the gastric mucosa the hemorrhagic spots are situated directly below the surface. The extravasation has taken place from the interglandular capillaries. These hemorrhagic areas are found to contain fairly numerous plague bacilli. It is perhaps possible that the portal of entrance of the plague bacillus in this case was the lungs. However, since no areas of consolidation were found and the infection of the lungs was quite moderate, the case has been classified as one of primary plague septicæmia.

ANALYSIS OF THE TWENTY CASES REPORTED AND SUM-MARY OF PATHOLOGICAL FINDINGS.

An analysis of the twenty cases of plague reported above shows that the total figure is made up of:

Group I.	Primary uncomplicated bubonic plague	Cases.
	Primary bubonic plague with secondary plague septico-	
	pyemia	4
III.	Primary bubonic plague with secondary plague pneumonia	1
IV.	Primary uncomplicated plague pneumonia	2
V.	Primary pneumonia with secondary septico-pyemia	1
VI.	Primary plague septicæmia	. 1

Sixteen of these twenty cases were of the bubonic type, three of the pneumonic, and one of the septic type. Of the bubonic cases there were:

		Cases.
Inguinal	buboes	11
0	buboes	1
Cervical		1
Cervicai	bubbes	4

The great preponderance of the bubonic type of plague cases is fully in accord with the observations made by the Indian Plague Commission on a very large amount of material; and the preponderance of inguinal buboes over cervical and axillary ones is likewise the rule among races and in localities where the people in general are barefooted. Of the cases of cervical buboes there were three found in children, among whom their occurrence is the most common, and one in a man of 63. Twelve of the 20 cases occurred in native Filipinos and 8 in Chinese, 15 in males, and 5 in females. The great preponderance of males over females is partly due to the fact that in one part of the affected population namely, the Chinese—the greater proportion consists of males. However, even in India the number of cases in males greatly exceeds that in females, though not in the proportion of three to one. As to age, the cases reported range from 5 to 63 years, which is in accord with the general experience gained on a large scale. The majority of our twenty cases—viz, thirteen—occurred between the ages of 20 and 40. Almost all of the plague victims came from the lowest walks of life of the native and Chinese population. One of the cases was that of a Chinese shopkeeper or merchant.

PORTAL OF ENTRANCE OF THE VIRUS.

As is well known, the portal of entrance of the plague virus can not always be found. In the sixteen bubonic cases there were seven in which even the slightest indication of the probable portal of entrance was absent (cases Nos. 1, 5, 6, 7, 8, 12, and 14). In the majority of the remaining nine cases of bubonic plague proper the portal of entrance was more or less unmistakably indicated. But in connection with this subject one must not forget that a secondary skin plague lesion might be mistaken for the primary portal of entrance. This is of course particularly liable to happen in cases in which but little, if anything, is known of the clinical history. Where a reliable clinical history can be obtained such a mistake is less liable to occur. In the three pneumonic cases, the portal of entrance of the virus was the lungs, because the changes in the bronchial glands were such as to destroy the probability of their being the first focus of infection. In case No. 20-primary plague septicæmia—there was no evidence of an intestinal origin; however, one might in this case suppose that the bacilli were inhaled, without causing a distinct pneumonia, entering the blood current almost immediately and in this manner producing a general septicæmia without any manifest focal localization.

PERIOD OF INCUBATION.

The period of incubation of plague, as a rule, is a short one. The Indian Plague Commission has compiled a table of cases in which the time of infection could be fixed beyond doubt, it being contracted during post-mortem examinations or during some similar manipulation of fatal human plague cases. From their table it appears that the period of incubation generally ranges from one to five days.

"While thus," the report of the Commission (Vol. V, p. 88) says, "no facts have been reported to us which establish that the incubation period may be prolonged beyond the period of five days, the limit fixed by the numerous cases detailed above, * * * we think that it is a matter of importance to point out, that where the plague first takes the form of pestis minor, and where afterwards pestis minor develops, owing to some subsequent loss of resisting power, into typical bubonic plague, there is a possibility of a considerable interval elapsing between the time at which the infection was contracted and the time at which the clinical symptoms became typical. Such cases, important as they are in the epidemiological study of the disease, as indicating one of the possible

but quite exceptional ways in which plague may be carried about to distant centers over sea, are, it must be observed, of extremely rare occurrence. One such case has, however, come unofficially to our knowledge in Poona."

THE LYMPH GLANDS IN BUBONIC PLAGUE.

In the majority of cases the portal of entrance of the plague bacillus is through the skin or the mucous membranes. We have already emphasized the fact that there is no case of human plague on record in which the entrance of the bacillus through the intestinal mucosa could be demonstrated beyond doubt. After the plague bacillus has gained access to the body, it generally travels through the lymph channels to the nearest gland or glands, where it then multiplies. Indeed, the multiplication of this organism in the lymph glands in most cases of bubonic plague is undoubtedly in excess of any other bacterial proliferation in any other infection, not excluding lepra. In the lymph glands the changes produced may vary from comparatively minor ones to those of the most profound degree. From a study of the lymph glands in the various stages of the infection it appears that the first change produced is vascular dilatation and engorgement and œdematous infiltration with a perivascular proliferation of the connective tissue elements. Even in cases of mild infection, one generally finds an increase of connective tissue at the hilum and usually marked vascular dilatation and ædema of the gland. The effect of the toxines and the other metabolic products of the proliferating bacilli clearly brings about an early damage to the vessel walls. However, this damage may not find a pronounced morphologic expression. From statements made in literature it appears that a well-marked hyaline degeneration of the vessel walls is always found. We can not confirm this observation, because we have frequently found an infected gland with vessels, the walls of which, to all intents and purposes, appeared to be morphologically intact. However, there always is noticeable a great dilatation and engorgement of the vessels, factors which, as we well know from general pathology, are perfectly sufficient so to damage the vessel walls as to render them pervious to the serum and to the corpuscular elements of the circulating blood. Hence, it is not necessary, in order to explain some of the tissue changes in plague, always to look for a degeneration of the vessel walls with grave morphologic changes.

condition of dilatation and engorgement of the vessels with general cedema of the gland tissues has been established, diapedesis of the red blood corpuscles occurs; so that, as a rule, a lymph gland infected to any extent with plague bacilli shows blood extravasation. When the infection has become very extensive and the number of bacilli very great, we generally encounter the most profound tissue changes in the glands, associated with extensive blood extravasation. To the naked eve a gland in this condition appears much swollen, more or less softened, and from dark scarlet to brownish-red, with yellow or yellowish-red mottling. Since the blood extravasation extends beyond the gland proper, the periglandular tissue is likewise of a hemorrhagic color. The tissues in the neighborhood of the gland are ædematous to a considerable extent. If the finer histologic changes of the gland are studied, it is found that the original tissue has frequently become necrotic; the differentiation into cortical and medullary portions and into follicles and cords is lost. The capsule of the gland has become loosened by ædematous and cellular infiltration, and the latter extends far into the periglandular tissue. Most of the original cells of the gland show evidences of nuclear disturbances (pyknosis, etc.) and of coagulation necrosis, and a large number of red blood corpuscles infiltrate the entire tissue and invade the periglandular areas. The vessels are greatly dilated and engorged and their walls are frequently loosened by hydropic swelling and cellular infiltration; or they may be in a condition of more or less complete hyaline degeneration. Not infrequetly one finds proliferated lymphatic endothelia in larger numbers in the infected glands; these endothelia may or may not show phagocytic properties, other cells or plague bacilli frequently being included in them. Plague bacilli are often present in the glands in dense zoogleal masses. From such a center of enormous infection the bacilli infiltrate the rest of the gland and the periglandular connective tissue in more or less continuous groups. In several of our cases we have seen in extensively infected and hemorrhagic glands, a perfectly homogeneous vacuolated hyaline material, which has a strong affinity for eosin and which we consider a derivative of degenerating, agglutinated red blood corpuscles. In fact, sometimes the derivation of the hyaline material from this source becomes so obvious that there can be no doubt of its origin.

FIBRIN FORMATION IN THE GLANDS AND IN THE OTHER ORGANS.

The homogeneous material just described is not true fibrin, and does not give the tinctorial fibrin reaction of Weigert. However, in plague true fibrin is found in the primary bubo in quite a number of cases as well as in the organs distant from the focus of infection. We encounter perfectly solid hyaline fibrin thrombi, or some vessels may be partly occluded by a hollow tubular wall thrombus or by a more or less open reticulum of fibrin. Such reticula, particularly in the bubonic glands and in the spleen, are also found in an extravascular location. Occasionally one sees threads connecting the intravascular and extravascular fibrin reticula. Sometimes fibrin is found, not in the shape of threads, but as granular, more or less solid material, always of course giving a typical Weigert's reaction. In the glandular buboes we observed fibrin in the shape of hyaline, vascular thrombi, or in the form of extravascular fibrin reticula, with or without a connection of the intravascular and extravascular deposits (in seven cases, Nos. 2, 9, 10, 11, 12, 15, and 20). Further fibrin formation was observed in the spleen in five cases (Nos. 5, 10, 11, 12, and 20), and in the lungs in three (Nos. 17, 18 and 19). Fibrin may be found in several organs in the same case (No. 10, in the primary bubo, the spleen, and the kidneys); or it may be found in the primary bubo and nowhere else (Nos. 1, 3, 7, and 17); or it may be encountered in the organs distant from the primary bubo without being present in the latter (Nos. 11, 12, and 15). In cases Nos. 18, 19, and 20, types of primary pneumonia and primary septicæmia, there were of course no lymphatic buboes present and no fibrin was observed in any of the lymph glands. Fibrin thrombi in the kidneys were found in seven cases. below "Hyaline Fibrin Thrombi of the Glomerular Capillaries.")

HEMORRHAGES INTO THE BUBO AND INTO THE DISTANT ORGANS.

In studing the histopathology of the infected lymph glands we encounter other changes besides fibrin formation. These changes are quite characteristic for plague and are found not only in the glands but in locations distant from the focus of infection.

The feature which is most characterictic of plague infection is its tendency to produce a general dilatation and engorgement of the vessels and subserous, submucous, parenchymatous, and interstitial hemorrhages. The most common locations of the latter are the epicardium, the pleura, the peritoneal covering of the stomach and intestines, and the mucosa of the stomach and intestines. There may also be subcapsular renal hemorrhages and blood extravasation into the pelves of the kidneys, the ureters, the bladder, and the male and female genital organs.

In bubonic plague proper hemorrhages into the buboes are very rarely absent. In only two of our sixteen cases of bubonic plague proper were they not present; in these death was clearly due not solely to the pest infection, but to a complication of circumstances (Case No. 8, Banti's disease; Case No. 16, ambulatory plague, terminating by embolism of the pulmonary artery). In fourteen out of sixteen cases the primary bubo showed a hemorrhagic condition. The heart in most of our cases showed subserous hemorrhages. In this organ they were found seventeen times, varying in size from a few small petechiæ to numerous and not infrequently large ecchymotic spots. The other internal organs, arranged in the order in which hemorrhages occurred in them most frequently are as follows: Stomach, fourteen times; intestines, eight; kidneys, six; bladder, three; and liver and ureters, each twice., Other organs in which hemorrhages were occasionally found are the gall bladder, the epiglottis, the esophagus, and the thymus. The most extensive and most widespread hemorrhages were found in Case No. 12namely, one of bubonic plague in a male Filipino with secondary septico-pyemia and metastatic dissemination of the bacilli. The primary inguinal bubo itself showed most extensive hemorrhages and an uninterrupted continuation of them from Scarpas' triangle up to the right kidney, involving in the area of blood extravasation all the glands, the periglandular tissue, the sheaths of the large vessels, and the perirenal, loose arolar tissue. The heart, the kidneys, the ureters, the bladder, the liver, the stomach, the esophagus, and the small and large intestines, all showed either subserous or submucous hemorrhages, or a combination of both; blood extravasation was also seen in the anterior mediastinum. On the other hand, Case No. 8, one of the primary inguinal uncomplicated bubonic type, did not show a single hemorrhage. This is the case in which we found a spleen weighing 865 grams and a cirrhotic liver. It is reasonable to suppose that this patient had no resistance to the

plague infection and succumbed speedily before the characteristic tendency to free hemorrhage could become manifest.

The Austrian commission, in particular, held that multiple hemorrhages in plague are dependent upon the direct presence of bacilli. However, this view is not adopted by most observers; agreeing with the majority of authors we are strongly opposed to it. In all of our cases we examined the hemorrhagic areas carefully and in the larger number of them found the petachiæ and ecchymoses free from them. Plague organisms were found only exceptionally in the hemorrhagic areas, and only in such cases as showed a septico-pyemic dissemination of the bacilli. To repeat, in the greater number of cases of the uncomplicated types of bubonic plague, we were unable to detect any bacilli in the numerous subserous and submucous hemorrhages.

The German commission was never able to find any bacilli in the juice obtained from the petechiæ; and several authors have reported the typical occurrence of hemorrhages in guinea pigs killed by dead cultures of plague bacilli. We have likewise succeeded in producing extensive blood extravasations in distant organs in guinea pigs killed by intraperitoneal injections of dead cultures. Hence, we must hold that the hemorrhages are entirely independent of the local invasion of living plague bacilli and are due to the toxines which have entered the general blood current.

PLAGUE AS A RULE NOT A GENERAL MICROBIC INFECTION.

In uncomplicated cases of bubonic plague, even when the infection of the glands is extensive, bacilli are generally not found in the vessels. In fact, one may observe an area composed almost exclusively of zoöglæal masses of bacilli, in which there is still found a vessel with a comparatively intact wall and with a lumen entirely free from plague bacilli. In the introduction we have already called attention to the fact that the results of our investigation do not justify us in classifying plague, in the strict sense of the word, as a general hemorrhagic septicæmia, but as a local infection with general symptoms and a general hemorrhagic toxemia.

From a histologic study of our material, we have come to the conclusion that a general dissemination of the bacilli from the glands is the exception and not the rule. Usually the infection

remains localized in a gland or in a group of glands. However, there does occur a final agonal dissemination of the organisms, which does not differ in character from that of other microorganisms in other diseases. In a number of cases an extension of the invasion by bacterial metastatic emboli does take place, but even in these instances there is no evidence that the plague bacilli multiply throughout the entire blood system, although we encounter either more or less numerous metastatic emboli, generally in the liver and the kidneys.

THE INFECTION OF THE SPLEEN.

However, one organ is perhaps infected in every case of plague—the spleen. The pest bacillus, even in uncomplicated bubonic cases, always gains access to its pulp spaces, and here it evidently finds conditions favorable to its multiplication.

In this respect plague may be likened to typhoid fever, in which we also have a primary infection of the lymphatic tissue (Peyer's patches) with an infection of the spleen which is never absent. Another parallelism between typhoid fever and plague may be found in a secondary infection of the lungs. Just as we find secondary typhoid pneumonia dependent upon the presence of enormous numbers of typhoid bacilli in the lungs, so we find secondary plague pneumonia dependent upon the presence of enormous numbers of plague bacilli in these organs.

That cases of plague are usually not complicated by a general septicæmic dissemination of the bacilli is also strongly indicated by the result of blood examinations on living human beings. (For an abstract of literature on this subject see Herzog and Hare: Does Latent Plague Exist, etc.?)

EXTENSION OF THE PRIMARY BUBO.

After a gland has once become infected by plague organisms and been invaded by enormous numbers of bacilli, it is usual for the other lymphatics of the same chain to suffer likewise; the latter then present the same gross and finer pathologic changes as the former. For instances, it may happen that the first gland to be invaded is a femoral one, from which the infection spreads from gland to gland until it reaches the retroperitoneal lymphatics high up in the abdomen. As in some of our cases, we may then find a

continuous chain of glands, swollen, softened, and hemorrhagic, extending from below Pupart's ligament to the kidneys and embedded in a completely hemorrhagic and gelatinous connective tissue. The same may be true of the axillary glands where the typical changes and the hemorrhages may extend from the axillary space into the thoracic cavity, and in the case of the cervical glands, from the most superficial cervical to the deepest submental lymph nodes and even in to the mediastinal glands.

THE OTHER LYMPH GLANDS IN PLAGUE.

Aside from the primary bubo with its hemorrhagic glands of the first, second, third order, and so on, the glands in general may present a greatly varied appearance, and we find all gradations from the most profound to very insignificant changes. In septico-pyemic cases metastatic emboli may be carried from the primary bubo into a distant gland, and there establish a secondary bubo, which does not differ materially either macroscopically or microscopically from the former. But even in uncomplicated cases, without septicopyemia, the distant glands may be much swollen, greatly softened, and highly congested, or they may practically show no changes at all. Only a few glands may be affected, or many of them may exhibit marked pathologic changes. In general, the rule holds good that the more marked the tendency to widespread, subserous and submucous hemorrhages, the more pronounced the swelling, etc., of the lymph glands, both superficial and deep. In plague pneumonia the bronchial glands may have the appearance of a primary bubo, or they may merely show some swelling, softening, and congestion, without any blood extravasation. The lymph vessels proper, aside from those situated in the infected glands themselves, are as a rule not much changed, excepting those which connect the different glands of the chain forming the primary bubo.

THE HEART AND THE CIRCULATORY SYSTEM.

As a rule the heart does not show any very pronounced changes in plague, aside from those which are found as common features in the various organs in this disease. The great frequency of subepicardial hemorrhages has already been pointed out. The coronary vessels are generally greatly dilated and engorged, and the smaller ones of both the visceral and the parietal layers of the pericardium are often distinctly visible as red tortuous streaks and The pericardial fluid is normal or somewhat turbid and occasionally more or less increased in amount. The left ventricle is contracted and the right one sometimes considerably dilated. We have never seen endocardial hemorrhages or endocarditic processes. However, Duerck has described a case of plague pneumonia with endocarditis verrucosa of the mitral valve, further complicated by a purulent leptomeningitis of the convexity. case was evidently one of septico-pyemic plague endocarditis. myocardium is frequently of good consistency and of normal color; although sometimes it is rather soft, flabby, friable, pinkish-yellow, and easily torn. Both sides generally contain chicken-fat clots and much dark, fluid blood. The great vessels present no changes peculiar to plague; but in Case No. 16, an ambulatory one, we found an embolism of the pulmonary artery. In general the veins and capillaries are dilated and engorged to such an extent that some of the internal organs, more particularly the lungs and the kidneys, are not only greatly congested but highly cedematous. If one considers the great dilatation and engorgement of the vascular system and the ædematous infiltration of the tissues met with in plague, one is inclined to think of a hydremia of the blood. Such a condition might easily be brought about in consequence of the vasomotor and secretory disturbances which exist in plague, namely, scanty or suppressed urine and dry skin on the one hand, with great thirst and a larger ingestion of fluid on the other. Whether a hydræmia of the blood really exists in plague is a point, which, as far as we know, has never been determined. This question could, of course, not be decided by a count of the corpuscles, but only by exact chemical methods for determining water and dry residue.

The dilatation and engorgment of the vascular system often shows the smallest veins in the serous and mucous membranes as distinct, more or less tortuous lines, while the overfilling of the capillaries in the membranes named produces a uniform deep hyperæmia. In areas of this kind petechiæ and ecchymoses are encountered. Microscopically the myocardium often does not show any marked changes. In other cases a moderate degree of fatty degeneration, pigmentary degeneration of the perinuclear zone, fragmentation or segmentation are met with. However, in general, the histologic changes of the myocardium are insignificant. We

have never been able to find any bacilli in the subepicardial hemorrhagic areas.¹

THE LUNGS.

The lungs, in uncomplicated cases of bubonic plague as well as in complicated ones, in fact in all cases of pest which we have examined, show great congestion and cedema, with a corresponding diminution of the amount of air contained in them. The external surface is generally of a deep purplish color; the cut surface discharges a large quantity of blood and serous fluid. The mucosa of the bronchi, the trachea, and the larynx is more or less swollen, congested, and reddened. Hemorrhages are found on the pleural surfaces and sometimes on the mucosa of the bronchial tract, including the larynx; in one of our cases they were found in the epiglottis. In cases of primary or secondary plague pneumonia we find interlobular foci in the lungs, varying in size from that of a mere point to that of a hazelnut. They are generally well consolidated and in color grayish-reddish-white to brownish-red. They are, as a rule, surrounded by a ring of intensely congested, deep reddish-brown pulmonary tissue. The areas of lobular consolidation may become confluent so as to produce a picture of complete lobar consolidation. We have not observed such cases, but others have established their occurrence.2 In one of ours the pneumonic area reached to the pleural surface, where it had produced a localized exudative, fibrinous pleuritis. In general it is found that if the areas of lobular consolidation are situated near the surface, the pleura in this region is somewhat prominent and uneven, and is quite well differentiated from the surrounding surface. In plague pneumonia the bronchial glands may or may not assume the character of primary buboes. They may be enlarged. greatly softened, and hemorrhagic, or they may show but very

¹ No mention of the myocardium is generally made in the microscopic reports of the twenty individual cases reported above. However, the heart and other organs not specifically mentioned, such as the suprarenal glands, the pancreas, male and female genital organs, etc., were examined microscopically in most of the cases. Since no changes of a marked character or of particular interest were found, these findings have not been given in detail but are only referred to in the summary.

² In case No. 18, the same which showed the localized fibrinous pleurisy, most of the left lower lobe was consolidated. So this case came very near being a lobar pneumonia.

little change. On microscopic examination we find that the alveoli are either filled with larger or smaller numbers of bacilli or with a cellular exudate consisting of leucocytes, alveolar epithelia, and erythrocytes, mixed with smaller or larger numbers of plague bacilli. The interalveolar septa are generally widened by an ædematous infiltration and by a swelling of the cells and fibers. The capillaries are greatly dilated and engorged; they may be free from plague bacilli or may contain metastatic emboli in complicated cases. Fibrin may be either present or absent.

THE SPLEEN.

Much emphasis has been laid upon the enlargement of the spleen in plague. Some observers have even gone so far as to state that this organ is always markedly enlarged in this disease and not infrequently very much increased in bulk. However, we have, among our post-mortem material four cases (Nos. 4, 5, 10, and 17) in which the spleen was not enlarged and two (No. 14, Filipinos sixty-three years old, and No. 18, Chinese twenty-seven years old) in which it was very small. On the other hand, most cases of plague show a considerable increase in the size of this organ, which then is generally about two to three times the bulk and weight of the normal one (Cases Nos. 1, 2, 7, 9, 12, 13, 15, 16, 19, and 20). In three cases (Nos. 3, 6, and 8) a very considerable enlargement was found, in one of which at least an increase of the organ to 20 by 12 by 7 centimeters and a weight of 865 grams, was clearly due to causes existing prior to the plague infection. One must not forget that splenomegaly is a very common finding in the Tropics; and hence if a very large spleen is encountered in a plague case the possibility of this being due to some other cause must always be kept in mind. We have taken great pains to search for the Donovan-Leishman bodies in those of our plague and in other post-mortem cases which showed extensive enlargement of the spleen, but we have never encountered them. This demonstrates, to a certain extent at least, that all cases of tropical splenomegaly can not be attributed to the Donovan-Leishman infection. In plague the enlarged spleen is generally, though not always, soft in consistency, dark purplish-blue externally, and dark brownish-red on the cut surface. On section the pulp generally protrudes and a large quantity of juice can usually be scraped off. The trabeculæ as a rule are distinct, but the follicles are commonly not very clear.

Why the latter are not readily distinguishable is easily explained by the microscopic examination of the organ, which frequently shows small follicles indistinct in outline, owing to the fact that the cells forming them are lost in the surrounding pulp spaces. The latter themselves are generally not distinct and are occluded by cellular elements, consisting of red blood corpuscles and nucleated leucocytes. In a considerable number of our cases the spleen gave evidence of an extensive proliferation of the endothelial cells of the pulp spaces. Sometimes great numbers of these are seen, many of them containing two or more nuclei. A number of them are phagocytic and include either other cells or plague bacilli. As already stated, in all of our cases the spleen showed an invasion with bacilli, so that we are much inclined to look upon this as a regular occurrence in plague.

THE GENITO-URINARY SYSTEM.

It had already been emphasized by Virchow that in plague the genito-urinary system often presents subserous and submucous hemorrhages. This is the common observation of all who have studied the pathology of the disease, and it is also confirmed by our work. As a whole the kidneys invariably show marked congestion, and even to the naked eye evidences of parenchymatous degeneration are never absent. On the cut surface there are seen uriniferous tubules which are greyish-white or grayish-yellow as well as dull, and engorged vessels, among which are the glomerular capillaries. The Malpighian tufts are usually observed as intensely red points, although at times they may appear as more or less solid, grayish-red masses. This latter appearance may easily be overlooked until our attention has been drawn to it by a microscopic examination, which fully explains why one might expect to see such solid, grayish-red, swollen glomeruli.

A microscopic change which has been described by all who have studied the kidney in plague is profound cloudy swelling of the epithelium of the uriniferous tubules, with the presence of granular or hyaline material in the latter.

HYALINE FIBRIN THROMBI OF THE GLOMERULAR CAPILLARIES.

However, a very characteristic change in the kidneys which we have found in seven of our twenty cases, has not, it appears, before been described, namely, a hyaline fibrin thrombosis of the glomeru-

lar capillaries. These capillaries may be entirely or partly occluded by genuine fibrin thrombi, which give Weigert's staining reaction. The thrombi are either solid or hollow tubular wall thrombi. Where they are found, it can generally be shown that they extend into the afferent and efferent vessels and beyond these into the intertubular ones. A careful study of the vessels fails to show any appreciable morphologic change of their walls. The vascular endothelium generally appears intact; occasionally some of the lining endothelium may be missing, but this is certainly not general, but rather exceptional. The fibrin thrombi are evidently formed independently of the presence of plague bacilli in the kidneys; because in most of the cases where we did encounter them in our material a very careful search for these organisms in sections failed to reveal them. However, in one case in particular there was a simultaneous occurrence of hyaline thrombi and bacillary emboli. In some places the bacilli were actually located between the vessel wall and the thrombus. However, since this was seen only in one case, it is very clear that it was merely a coincidence; a causal nexus between the bacilli and the thrombi did not exist. The very characteristic hyaline fibrin thrombosis of the glomerular capillaries does not appear to be found frequently in acute infectious diseases in which the kidneys are greatly involved, nor, in fact, in any form of acute or chronic nephritis.

Welch, however, appears to consider it as not at all an uncommon occurrence, and makes the following statement in regard to it: "Capillary hyaline thromboses are common in the lungs in pneumonia, and in hemorrhagic infarcts. In general infective and toxic states they may be present in the liver, the lungs, and above all, in the kidneys. 1 The most striking example of this form of thrombosis with which I am acquainted is encountered in renal capillaries, chiefly of the glomeruli of swine dead of hog cholera; or of animals infected with the hog cholera bacillus. In extreme cases there is complete anuria, and it may be impossible to force more than a minimal amount of injecting fluid into the renal vessels. Section stained with Weigert's fibrin stain look as if the capillaries had been injected with Berlin blue. Ribbert found similar hyaline thrombi in the kidneys of rabbits inoculated with S. Pyogenes aureus. I have repeatedly found them in various experimental infections and in human infections. They occur in eclampsia. Bacteria are not necessarily present, so that toxines are probably the underlying causative factor, and for this there is experimental evidence."

It must be borne in mind that the term "hyaline thrombosis of the

¹ Italics our own; not in the original.

glomerular capillaries" is frequently very loosely used by authors who have written on changes of the kidneys in infections and it generally refers to an ordinary hyaline degeneration and not to a true hyaline thrombosis. In fact, a very careful study of the literature impresses one with the conviction that the latter is very rare. It has been mentioned with some emphasis in connection with only one renal affection, namely, late post-scarlatinal nephritis. Our attention was first called by Klebs to the changes in the glomeruli in diseases of the kidneys who introduced the term glomerulo-nephritis and who stimulated further research into this histo-pathologic change. He states that after scarlatina, the kidneys are found either slightly or not at all enlarged and very rich in blood, and the glomeruli appear as small, whitish points, which in sections are seen to contain little blood and are darker and more cloudy than the uriniferous tubules. However, neither Klebs nor Langhans, who studied kidneys with glomerular lesions in twelve cases of scarlatina, say anything about hyaline fibrin thrombi in the glomerular capillaries. The latter states expressly that the fibrin cylinders in the tubules are composed of a hyaline material which is not identical with fibrin. Boehm, Goemans, Fichera, and Scafiddi 1 have recently published contributions to the pathologic histology of the glomerulus, but they have not seen any fibrin thrombosis of the glomerular vessels. The last two authors describe hyaline degenerations of the glomerular capillaries and state that in the kidneys, with changes in the glomeruli in the kidneys, there frequently occur affections of the capillaries. The process begins in some loops, which become glass like and transparent and take certain stains homogeneously. The process then spreads to several which become fused and so form a homogeneous mass poor in nuclei. The few nuclei left show profound disturbances, such as karyolisis or karyorrhexis. In still more advanced cases one sees, in place of the glomerulus, a body smaller than the smallest glomerulus, which is globular and bounded by a capsular membrane. The body itself consists of a homogeneous, transparent, uniformly stained, hyaline mass, which shows no capillaries at all or only traces of them, and in which no more capsular space can be recognized. Nuclei, if present at all, are either small or poorly stained, shrunken, pyknotic, or fragmented. The cause of the necrosis and hyaline degeneration of the glomerular capillares may probably be attributed in this case, as in others to profound disturbances of nutrition of the vessel walls and to obliteration of the capillaries which prevents the blood circulation. Fichera and Scafiddi expressly mention that the process described is different from true capillary thrombosis as it has been observed in post-scarlatinal nephritis. Pearce investigated the histopathology of the kidney in twenty-three cases of scarlatina, but did not find glomerular thrombosis in any of them. Hansemann microscopically examined the kidneys in 120 cases with special reference to the changes found in the

¹ Fichera and Scafiddi's quite exhaustive study contains a complete list of the literature and quotes 110 articles on the normal and pathologic histology of the glomerulus.

Malpighian bodies. His material included the following diseases: Nephritis, after cold, parturition, scarlatina, diptheria, measles, croup, pneumonia, typhoid, erysipelas, puerperal fever, endocarditis ulcerosa, malaria maligna, constitutional syphilis, tuberculosis, eclampsia, atrophia encephalitis neonatorum, cholera nostras, epidemic meningitis, empyema without tuberculosis, carcinoma, pernicious anemia, leukemia, sunstroke, and chlorate of potash-phosphorus-lead and arsenic poisoning. In none of these affections did he ever find a hyaline fibrin thrombosis of the glomerular vessels; in fact, he does not mention it, though he speaks of amyloid degeneration. Posner is probably the first writer specifically to mention the presence of typical fibrin in the kidneys, however, not in the glomeruli but in the interior of the tube casts, found in the kidneys of rabbits after the ligation of the renal artery. Israel observed reticula of fibrin in the convoluted tubules, and solid fibrin masses in the capsular space of the glomeruli in experimental anemic necrosis induced by ligation of the renal artery. He evidently did not see fibrin thrombi in the glomerular capillaries, because no mention is made of such an occurrence. Engel reports the observation of five cases of chronic nephritis accompanied by glomerulitis with the presence of fibrin threads in the capsular space. These threads gave a positive Weigert's staining reaction. Nothing, however, is said about the presence of fibrin in the glomerular capillaries, Kahlden describes a case of post-scarlatinal nephritis with complete anuria, in which he observed an obliteration of the glomerular capillaries by a fibrillar and granular material which he considered to be typical fibrin. The glomerular fibrin thrombi were continued into the vasa afferentia and efferentia and frequently beyond these into the arteriola recta. However, Kahlden was not successful in staining the thrombi satisfactorily by Weigert's method, as his tissues had all been fixed in Flemming and in Mueller's fluids. Friedlaender, according to the preceding writer, previously described a type of postscarlatinal glomerulo-nephritis which manifests itself from three to four weeks after scarlatina and which is characterized by œdema, albuminuria, oliguria, or even anuria. The microscopic examination of the kidneys in such cases shows glomerular capillaries changed into solid, sausagelike masses, making it scarcely possible to differentiate the wall of the capillary from its contents. Whether Friedlaender looked upon this thrombotic material as fibrin does not appear. Ernst found fibrin in the capsular space of the glomeruli and also in the interior of the hyaline tube casts in cases of nephritis. No mention is made of fibrin thrombi in the glomerular vessels. Ribbert, speaking of glomerulo-nephritis in one place and hyaline thrombosis in another, mentions hyaline fibrin thrombi as occurring occasionally in the glomerular capillaries. Tschistowitsch, one of the most recent writers on obliteration and hvaline degeneration of the glomeruli, does not mention hyaline fibrin thrombosis.

From a review of the literature on the subject of glomerular changes in nephritis it would certainly appear that hyaline fibrin thrombosis of the glomerular capillaries is a rather rare occur-

rence, which has been more fully described only in connection with late post-scarlatinal nephritis and with swine plague by Welch. It would probably be a thankless task here to attempt an explanation of the formation of hyaline fibrin thrombi in the vessels of the kidneys and of other organs, particularly in the infected lymph glands in plague. Indeed, such an attempt at explanation would open up the whole question of blood coagulation and of fibrin formation inside and outside the blood vessels. Loeb has quite recently published an experimental contribution to this subject With reference to thrombosis and the formation of fibrinous exudates he expresses the view that after the removal of the vascular endothelium the specific substances causing coagulation are extracted from the tissues. The ferments acting upon fibrinogen, held in colloidal solution, precipitate it in the form of fibrin. However, in most of our renal sections, in which hyaline fibrin thrombosis has been seen in the glomerular vessels, the vascular epithelium was to all intents and purposes morphologically intact and well preserved. It is of course not at all unreasonable to suppose that the plague toxines acting as they appear to do in particular upon the kidneys, so damage the vascular epithelium that it becomes pervious to the coagulating ferments, before any morphologic changes indicate a serious functional disturbance.

THE URINE IN PLAGUE.

That the kidneys are profoundly affected in plague is illustrated not merely by the findings on the post-mortem table and by microscopic examination of the sections but by the clinical history of the disease.

The German commission reports that it found albumen in the urine in most cases of plague in which a urine examination was made, while hyaline and granular casts and few or numerous blood corpuscles were seen occasionally. Calmette and Salembini state that it is only in grave cases that the urine is diminished or entirely suppressed. They sometimes saw bloody urine, and always found it very acid and with traces of albumen. Aoyama states that the albuminuria is generally moderate. Bitter found albumen in most cases, generally very moderate in amount; but in fatal cases it was invariably present. Yamagiwa found albumen only rarely. The Austrian commission examined the urine in forty-five cases and found albumen present in all of those where repeated examinations were made. The most extensive urine examinations in plague have been made by Cathorn in India. She found albumen absent in only 24, or 7 per cent, out of 341 fatal cases; while in 256 nonfatal cases it was

absent in 64, or 25 per cent of them. In some of our cases, with a septico-pyemic dissemination of the virus, we have found an extensive infection of the kidneys with plague bacilli; and the conclusion is justified that the bacilli must be abundant in the urine in such cases. In fact in articles on plague one frequently finds the statement that the urine generally contains bacilli; yet exact observations on this point have thus far been very meager.

The German commission reports that it found plague bacilli in the urine in only two cases. The urine both from plague patients and from post-mortem cases usually was found free from these organism. The Austrian commission frequently found numerous plague bacilli in the kidneys, but encountered them in only five out of seventeen different specimens of urine obtained post-mortem from plague cases. The Indian commission examined sixty specimens of urine by cultural methods and found the plague bacilli three times. Five other specimens were examined by inoculation into guinea pigs; in one case only did an animal develop plague.

These results obtained from the bacteriologic examination of urine in plague are well in accord with our own histologic observations. The bacilli were as a rule found in the renal tissue only in moderate numbers and in such situations that their appearance in the urine was not at all likely. Only when found in the capsular space of the glomeruli may we safely suppose them to have been present in the urine also. However, since one can not know intervitam whether an infection of the kidneys exists or not, it is well always to treat the urine in plague as a possible source of infection. The same rule applies to feces, which likewise, as the histologic study of the stomach and intestines teaches, may sometimes contain plague bacilli.

However we may here insert that the presence of plague bacilli in the feces must be still more exceptional than their appearance in urine.

The German commission was never able to demonstrate them in the feces either by culture or by animal inoculation. The Indian commission, after giving a detailed account of its work on the attempted detection of plague bacilli in the feces, says: "The results of the above series of examinations may be summarized in the statement that the plague bacillus as yet has not in any case been isolated from human feces. Such a negative result is, however, of very little account in view of the fact that it is, as we have seen, a matter of extreme difficulty to isolate by cultural methods the plague bacillus from a material such as feces, in which the bacillus coli and an infinity of other bacteria are to be found."

The pelves of the kidneys, in intensely hemorrhagic cases, occasionally contain a considerable amount of blood. In such cases the

bladder may likewise contain bloody urine or even blood coagula. Like the mucosa of the pelves, that of the ureters and the bladder occasionally shows petechiæ and ecchymoses.

The suprarenals are generally somewhat swollen, enlarged, softened, and congested, and darker in color than normal. We have not found any marked histologic changes in them, aside from dilatation and engorgement of the capillaries, conditions which are particularly noticeable in the interfascicular capillaries of the cortex.

THE LIVER AND THE GALL BLADDER.

The liver is generally of normal size, though it may be slightly enlarged. In those cases where we have found a decrease it was clearly not due to the plague infection but to morbid conditions previously existing. The capsule is smooth and tense; its external color is deep purplish-blue or purplish-pink. Evidences of a minor or more pronounced degree of fatty degeneration are rarely missing. Alternating with the general purplish color we find areas which are grayish-white or grayish-yellow. The cut surface shows a brownish-red or ocher-yellow color according to the degree of fatty infiltration and degeneration, and the veins discharge much blood. In septico-pyemic cases we encountered necrotic, soft, grayish-white foci. These may also shine through the surface and may have been noticed before the organ is cut into. The liver lobules are either distinct or more or less indistinct; and their boundaries may frequently be even more distinct than under normal conditions, which is due to the fact that interacinous, periportal, inflammatory foci are often encountered in the liver in cases of plague. They were found in cases Nos. 1, 2, 3, 7, 8, 9, 10, 14, 16, 17, and 20. The microscopic examination, aside from these foci, further shows dilated, engorged capillaries. The parenchyma cells are finely or coarsely vacuolated or in a condition of cloudy swelling or more or less complete granular degeneration. In uncomplicated cases of bubonic plague the parenchyma cells are not profoundly changed; but in septico-pyemic cases the change is generally quite profound. The necrotic foci, if present, contain large numbers of bacilli and frequently show capillaries more or less completely occluded by metastatic emboli. The cells in this neighborhood are in a condition of complete coagulation necrosis, which extends beyond the groups of bacilli into the adjacent tissues. Phagocytic, proliferated endothelia are sometimes seen in the liver.

The gall bladder is generally normal; but it may sometimes be profoundly changed, showing numerous subserous hemorrhages and a completely ædematous and almost gelatinous condition of the wall. The bile itself varies from a normal consistency and golden-yellow color to a pitchy condition and almost black color. The serosa of the gall bladder, like that of the liver, occasionally shows extensive ecchymotic spots.

We have not encountered any marked macroscopic or microscopic changes in the pancreas.

THE STOMACH AND THE INTESTINES.

The stomach and the intestines are generally markedly affected in plague. Petechiæ and ecchymoses are found both in the serosa and in the mucosa. Particularly the gastric mucosa is generally in a hemorrhagic condition. The petechiæ often extend into the duodenum and occasionally even into the esophagus. Microscopically, the mucosa of the stomach always shows greatly dilated interglandular vessels and frequently hemorrhages between the glands and into the uppermost layers of the mucosa or even upon its free surface. Plague bacilli were found in these areas only in septico-pyemic cases. The upper layers of the mucosa are often necrotic; and the cells of the peptic glands, even where the membrane is still fairly intact, show evidence of nutritive disturbance (multinuclear cells). The small and large intestine in general frequently show a great congestion of the mucosa and serosa with petechiæ and ecchymoses. In one case submucous blood cysts were found in the large intestine; these were probably due to the plague infection, as other, higher parasites could not be demonstrated in them. In a considerable proportion of our material the intestinal follicles were more or less swollen, viz, moderately in cases Nos. 6, 7, 15, and 17, and markedly in cases Nos. 1, 5, and 12, and highly in case No. 2. In the other cases the intestinal lymph follicles were normal.

STEPS IN ESTABLISHING THE POST-MORTEM DIAGNOSIS OF PLAGUE.

We shall now mention briefly, but systematically, all observations and experiments which will in a given case of suspected fatal plague enable us to come to a definite and conclusive diagnosis. However, it must be borne in mind that there are some exceptional cases in which a diagnosis is impossible. When a plague infection has run its course without having in itself killed the patient, he may nevertheless succumb to a secondary pyogenic complication, to parenchymatous degeneration of the internal organs or to exhaustion due to toxemia, any one of which may carry off the victim after the plague bacilli have disappeared from the primary bubo and from the system at large. Of course such exceptional cases are of no great epidemiological importance, since a spread of plague can not occur if no more bacilli are present. In the great majority of fatal cases of human plague the post-mortem diagnosis, to one familiar with the disease and with the ordinary technique of bacteriological investigation offers no particular difficulties. The diagnosis of plague in rat cadavers which are much decomposed is a matter of much greater difficulty, but in this article this is a subject with which we not concerned.

The report of the Indian Plague Commission (Vol. V, p. 442) gives the following description of the body of a patient dead of plague:

The body, if undisturbed, is stated to be generally lying on one side, with the knees flexed, and the head slightly bent on the chest. skin in dry, and in a few cases petechiæ and purpuric spots may be seen; the muscles are soft, as the rigidity of death is delayed; the features retain a fixed, anxious appearance; the eyes are half closed and sunken, with the pupils dilated; and the tongue is swollen, and although it sometimes has the appearance displayed during life, not unusually it is covered with a dry, almost horny dark or yellowish-brown and cracked fur. In pneumonic cases, the body seems especially shrunk and collapsed and has a livid aspect, and blood-stained sputum is often found adherent to the lips. Our attention was drawn by Captain Elphink, I. M. S., to the existence of edema, extending over the front and sides of the chest, the abdomen, and the upper part of the arms, which had been observed by him and by Captain Chaytor-White, I. M. S., in all plague corpses, and was believed to occur immediately before death. If this condition is generally present, it would be valuable as an aid in determining if death had been caused by plague, and especially in cases where no buboes are present. It has not, however, been observed by the great majority of those who in India have had the opportunity of seeing large numbers of fatal cases, both before and after death.

This guide to the post-mortem diagnosis of plague is evidently not intended for the pathologist performing a necropsy, but for the general practitioner or the layman-sanitary inspector of India. But we seriously doubt whether anybody will be materially assisted in a plague diagnosis by so vague a description as that given

above. The general œdema which is mentioned we have never seen in any of our plague cases.

The following are the steps in a systematic plague necropsy:

- (1) External inspection of the body.—As the most characteristic points brought out by the external inspection of a body dead of plague we would mention the following: The bubo is generally present in the femoral, the axillary, or the cervical region, and consists of a rather firm, doughy swelling over which the skin is generally adherent while the surrounding tissue is markedly edematous. In the neighborhood of the bubo may be seen an ulcer or a carbuncle with extensive necrosis. As a whole the surface of the body is frequently cyanotic; skin eruptions, petechiæ, and ecchymoses are commonly observed. A dark, blood-tinged, foamy, serous fluid always oozes from the nostrils of a plague body when it is turned over. This is due to the great congestion and ædema of the lungs, which we have never failed to observe in any of our cases.
- (2) The dissection of the body shows the multiple, interstitial subserous and submucous hemorrhages, the general congestion of the organs, the spleen tumor, the parenchymatous degeneration of the kidneys, and the lobular consolidation in pneumonic cases, described in detail above.
- (3) During the post-mortem examination inoculations of agar and salt-agar tubes or plates and gelatin plates are to be made from the bubo, or as the case may be, from the consolidated areas of the lungs, the spleen, the heart's blood, and other organs, particularly if they show areas of focal necrosis.
- (4) Preparations of smears from the same locations are to be made on slides, which as soon as air dry are immersed in absolute alcohol.
- (5) Pieces of tissue from the primary focus (lymph glands or lungs), from the spleen, and from the necrotic foci, are to be collected and placed in sterile, empty test tubes or Petri dishes.
- (6) Pieces of tissue are to be fixed in Zenker's solution for the subsequent histologic examination.
- (7) Animal inoculations.—After the termination of the autopsy the pieces of tissue preserved in sterile receptacles are to be used for inoculation into guinea pigs. A number of these animals are infected by the cutaneous method (Gohn and Albrecht) by rubbing some of the juice from the bubo (or lung) and the spleen on the

shaved abdomen. Other animals should receive subcutaneous and intraperitioneal inoculations of the suspected material triturated with sterile physiologic salt solution.

(8) After twenty-four hours the tube and plate cultures are to be examined with the naked eye, with the aid of the magnifying glass and in stained cover-glass preparations. The animals likewise are to be examined. Some of those which have received intraperitoneal injections are liable to succumb to the plague infection after twenty-four hours.

Martini has worked out a special method in the examination of guinea pigs infected cutaneously. Since this method often enables us to make a definite diagnosis in doubtful plague cases after forty-eight hours, it is to be recommended. His method is as follows:

- (a) The material suspected of containing the plague bacilli is triturated with about three times the quantum of sterile bouillon. This emulsion is rubbed with the back of a knife on the shaved abdomen of several (five or six) guinea pigs.
- (b) After twenty-four hours the guinea pigs are examined for swollen lymph glands, and if any are found, their juice is drawn with a sterile syringe and inoculated into agar tubes, and also examined in smears.
- (c) If what appear to be plague bacilli are found in the smears, two rats are inoculated intraperitoneally with the juice obtained from the enlarged gland of the guinea pigs.
- (d) The cultures obtained from the juice are examined macroscopically and microscopically and tested with an antiplague serum of known agglutinating power.

The agglutination test can not be made, it should here be stated expressly, with antiplague sera selected "at random," because some have little or no agglutinating power, as has been repeatedly pointed out by several observers, including the Indian Plague Commission. (Vol. V, p. 68 of report.)

Kolle and Martini recommend the desiccated antiplague serum of the Pasteur Institute for the agglutination test. The dry fine powder is first dissolved in ten times its weight of sterile distilled water (1 gram to 10 cubic centimeters. The solution is complete after several hours, whereupon the further dilutions necessary for the tests can be prepared. It is found that this dry serum will agglutinate genuine plague cultures, and only those, in dilutions of from 1:1,000 to 1:6,000. The less virulent the plague culture the greater the agglutinating powder of the dry serum; and vice versa, the more virulent the cultures, the lesser the agglutinating power.

Zlatogoroff, who studied the question as to how long plague bacilli remain alive and virulent in cadavers under various conditions of temperature and moisture, maintains that the cutaneous method of Gohn and Albrecht for infecting guinea pigs, is absolutely reliable only as long as the organs do not contain any considerable number of putrefactive and

other microbes. For diagnostic purposes in the case of old cadavers he therefore recommends a pernasal inoculation, which he practiced in the following manner: The lower part of one of the nasal cavities of a guinea pig is slightly wounded with a pair of sharp forceps or a needle, however, in such a manner that very little blood is drawn. A small piece, preferably of the spleen, is then grasped in fine forceps and introduced into the side where the slight trauma has been produced, after which it is well rubbed into the masal mucosa by rotatory motions of the forceps. This method is certainly somewhat objectionable on account of the danger of inducing a fit of sneezing in the animal operated upon, with uncontrollable dissemination of the plague material. Zlatogoroff himself considers this energetic procedure a "misstand," as he expresses it, but he confesses that it is indispensable, as otherwise the test may be negative in the presence of virulent plague bacilli. It also appears that this author has not ruled out the possibility of producing by his method in guinea pigs a disease which might simulate plague, a fallacy which, according to all the testimony obtained in this matter, is impossible by the cutaneous method. Zlatogoroff's investigation, at any rate, was not made with material derived from human cadavers, but with pieces of organs from the decomposed bodies of animals infected experimentally with plague. Since his publication has appeared after our report was practically finished, we have not taken an opportunity to try his method, and hence can not recommend it.

We do not wish to create the impression that all the cultural and inoculation experiments detailed above are necessary to arrive at a reliable diagnosis of plague in each suspected case. On the contrary, one familiar with plague can generally make a diagnosis after the termination of the necropsy, as soon as the smears from the organs have been examined microscopically. But in obscure cases all of the steps mentioned above may be necessary, and none of them should be omitted when a first-suspected case is to be diagnosed in a plague-free locality where an incontestable diagnosis is a necessity in justifying the protective and restrictive measures which should be instituted in order to prevent the spread of an infectious disease which at times many assume such destructive pandemic proportions.

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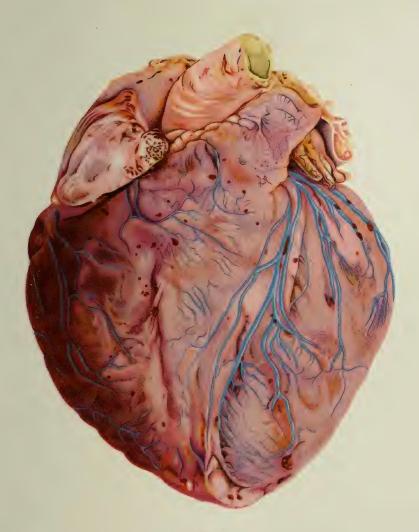
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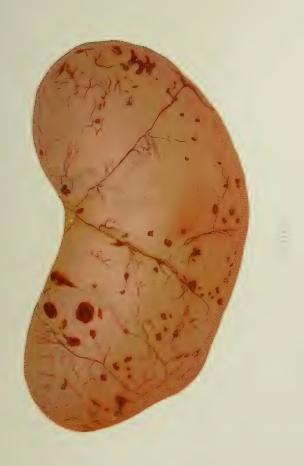


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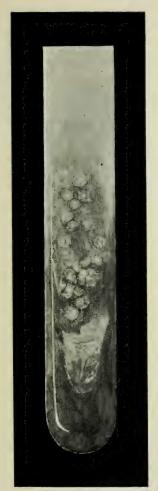
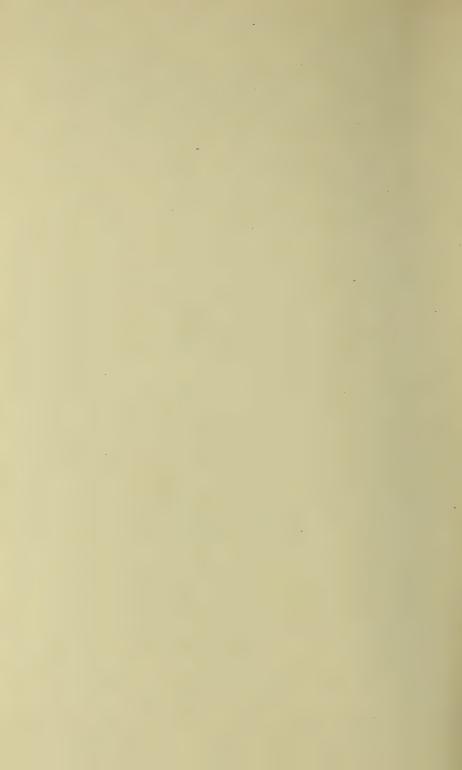


Fig. 6.



Fig. 7.



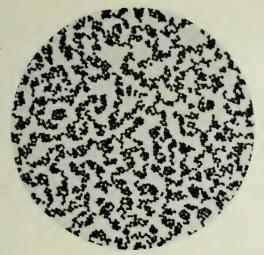


Fig. 8.







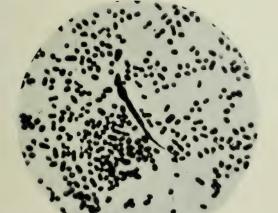


Fig. 11.



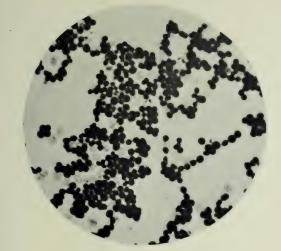


Fig. 12.

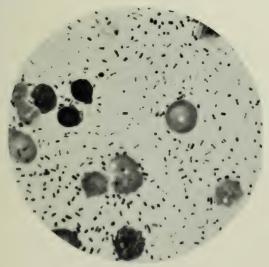
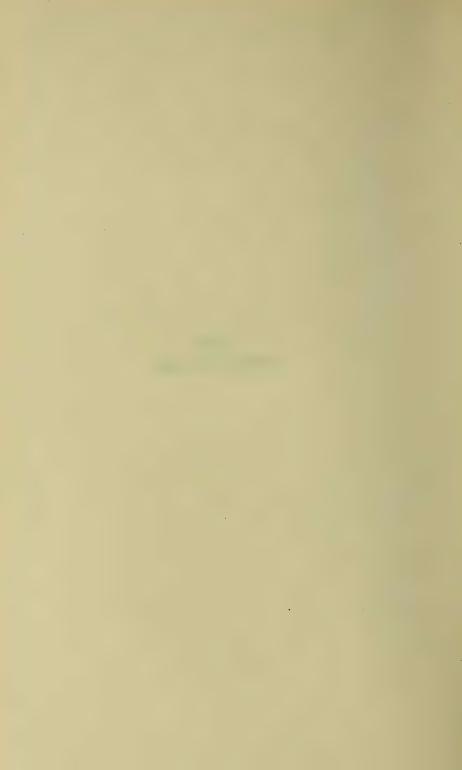


Fig. 13.



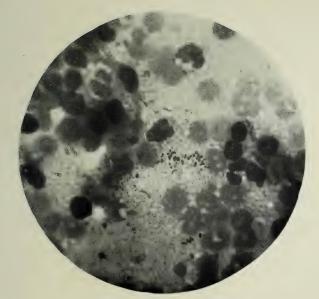


Fig. 14.



Fig. 15.



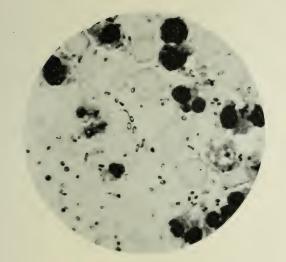


Fig. 16.



Fig. 17.



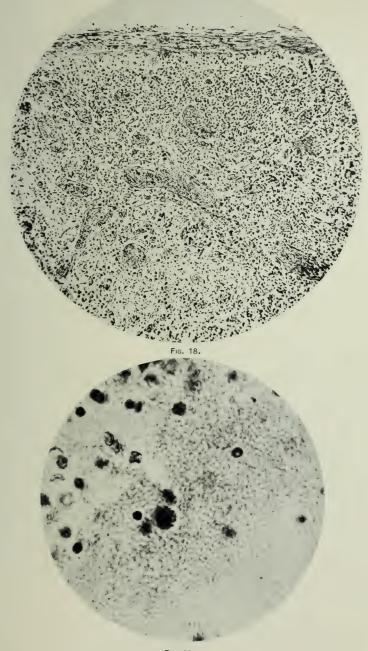


Fig. 19.



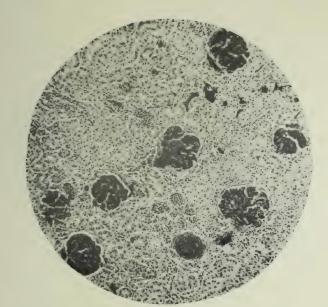
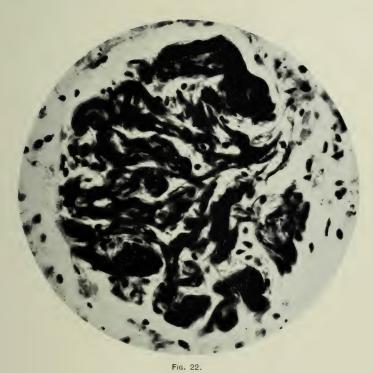


Fig. 20.



Fig. 21.











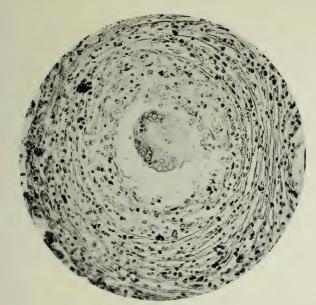


Fig. 24.

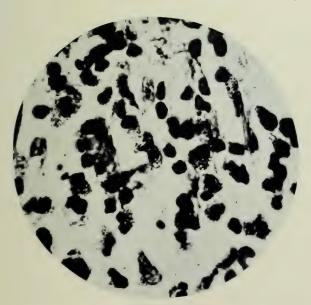


Fig. 25.





Fig. 26.

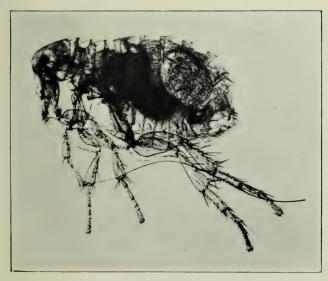
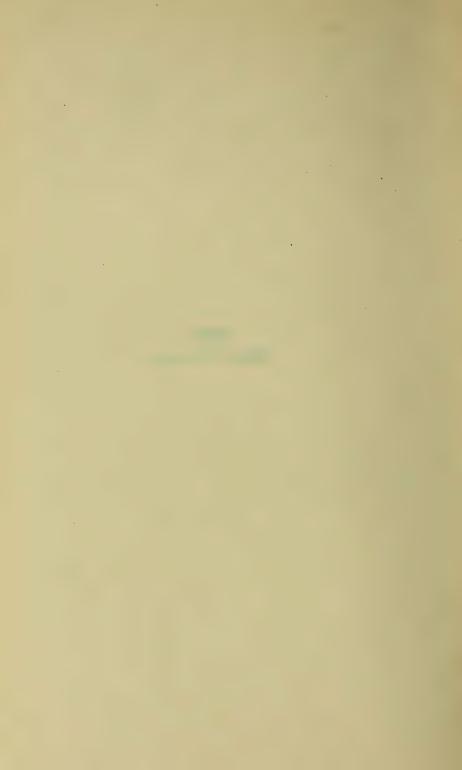
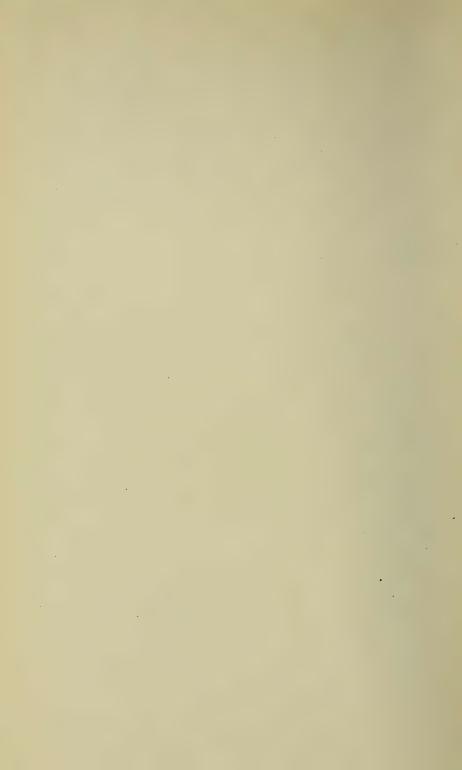
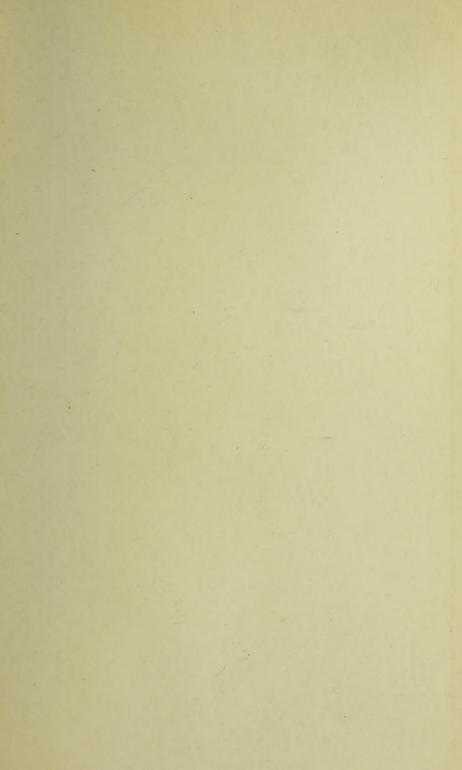


Fig. 27.













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